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Denitrification and its Relation to Soluble Carbon

by

© Marcia Amelia Monreal

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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To Carlos, Gilda and Elisa

Abstract

Organic matter is necessary as a reductant in denitrification. The supply of readily decomposable organic matter or water soluble organic carbon (WSOC) provides a good index of the soil potential for denitrification. Reports in the literature indicate an important role for the water soluble organic carbon pool, however, they do not demonstrate the controls and mechanisms supplying carbon to the soluble pool.

Experiments were performed in samples of Ap horizons from a Black Chernozemic soil (Malmo SiCL) and a Gray Luvisolic soil (Breton L) of the Province of Alberta, to examine some of the mechanisms supplying carbon to the water soluble pool; the relative magnitudes of assimilatory and dissimilatory NO_3^- reduction; the relationship between changes in WSOC content of soil and NO_3^- losses in saturated soils; and the effect of high NO_3^- or NO_2^- concentrations on denitrification.

Results indicated that the WSOC contents of the Malmo and Breton soils were significantly different (at the 1% level) and that extraction of the water soluble organic carbon was described by a modified Freundlich isotherm. When air-dried soil samples were incubated at field capacity for seven days, 158 and 45 $\mu g\ g^{-1}$ of WSOC were removed from the Malmo and Breton soils, respectively.

Microbial immobilization of NO_3^--^{15}N during 15 days under saturated conditions accounted for only 5% of the

NO_3^--N losses, so that denitrification appears to be the main mechanism of ^{15}N losses. The greater denitrification capacity of the Malmo rather than the Breton soil was consistent with its greater supply of WSOC. Denitrification ceased in the Breton soil when WSOC content dropped below 34 $\mu g\ g^{-1}$ soil. When glucose was supplied to the Breton soil high NO_3^--N losses prevailed. This suggests that the WSOC was limiting denitrification in the Breton soil. Although extensive NO_3^--N losses occurred in the Malmo soil, these losses ceased when WSOC content dropped below 114 μg C g^{-1}soil. The addition of a high dose of NO_3^--N (1500 μg N g^{-1}soil) to the Malmo soil inhibited denitrification and NO_2^--N accumulated. In the Breton soil, the application of NO_3^--N plus NO_2^--N (over 300 μg N g^{-1}soil) inhibited denitrification as well. C/N ratios calculated with the amount of WSOC consumed per unit N denitrified confirmed expectations that a greater amount of WSOC was consumed when N was in the NO_3^--N form than when it was in the NO_2^--N form.

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1. Introduction

For many years interest in denitrification has been due not only to the intrinsic nature of the reaction but also to its potential significance in agricultural production. This interest has been stimulated by the possibility of depletion of nitrogenous sources for crop production, by increased costs of nitrogen fertilizers, and by the practical necessity of avoiding nitrogen losses and maintaining a reasonable nitrogen level in the soil.

Moreover, this process has received additional attention because of its possible contribution to increased atmospheric concentration of nitrous oxide(N_2O) and destruction of atmospheric ozone (O_3)(Crutzen, 1974).

On the other hand, it has a considerable application in the removal of unwanted nitrogen from industrial wastes and domestic sewage (Delwiche and Bryan, 1976).

In Alberta, losses of soil nitrogen and fertilizer N have been reported to occur at the beginning of spring when the soil is completely saturated with water (Nyborg and Leitch, 1979). Using ^{15}N labelled fertilizer, Malhi (1978), concluded that N losses in early spring were almost exclusively through denitrification.

The objectives of this investigation were to study the following aspects of denitrification: i) the mechanisms supplying organic carbon to the soil solution; ii) the extent of nitrate losses by bacterial immobilization, relative to denitrification under anaerobic conditions; iii)

the relationship between NO_3^--N losses due to denitrification and the water soluble organic carbon content of soils; and iv) the influence of NO_3^--N and NO_2^--N on NO_3^--N losses.

2. Literature Review

2.1 Description of the Denitrification Process

The concentration of NO_3^- ions in soil is determined in part by simultaneous rates of production and consumption due to the activity of various soil microorganisms. During denitrification, nitrate is used by microorganisms as the terminal hydrogen acceptor following oxidation of organic compounds or in the oxidation of inorganic compounds, such as H_2S and H_2 ; giving rise to compounds at a lower stage of oxidation than NO_3^- (+5) such as NO_2^- (3+), NO (2+), N_2 (0), and NH_4^+ (3-) , when other more suitable acceptors are not supplied at a rate sufficient to meet the demands of these organisms. Consequently, denitrification in soil is principally confined to conditions of limited oxygen access together with sufficient hydrogen donors (Woldendorp, 1963).

In general, two different types of nitrate reduction are distinguished: assimilatory nitrate reduction and dissimilatory nitrate reduction, nitrate being used as a terminal hydrogen acceptor in both cases. During assimilatory reduction, NO_3^- is incorporated into cell materials. During dissimilation, NO_3^- is merely used as a hydrogen acceptor when other more suitable acceptors (like oxygen) are lacking or are not supplied at a sufficient rate.

2.2 Enzymic Mechanisms

When conditions are aerobic, oxygen is normally the electron acceptor for the reduced pyridine nucleotide carriers generated during intermediary metabolism; the cytochrome system, or " end respiration system", acts as a final link between reduced carrier and oxygen. When nitrate acts as an electron acceptor in place of oxygen, the complex reduced pyridine nucleotide is reoxidized at the expense of nitrate reduction and the action of nitrate reductase. Nitrate reductase is not a single enzyme but rather a complex enzyme system involving FAD, cytochrome c, and molybdenum (Campbell and Lees, 1967). At low oxygen concentrations a branch occurs at the cytochrome b level of the oxygen-terminated electron transport chain, and c-type cytochromes, synthesized for a nitrate terminated system, are utilized (Delwiche and Bryan, 1976). The cytochrome c donates its electrons (via a molybdenum-containing enzyme) to nitrate. The pathway can be represented by the following sequence:':

$$PNH + H^+ + FAD \longrightarrow FADH_2 + PN^+ \quad [1]$$

$$FADH_2 + 2\ \text{cyt. } Fe^{3+} \longrightarrow FAD + 2\ \text{cyt. } Fe^{3+} + 2H^+ \quad [2]$$

$$2\ \text{cyt. } Fe^{2+} + 2H^+ + NO_3^- \text{-(molybdenum enzyme)} \longrightarrow \quad [3]$$

$$\longrightarrow 2\ \text{cyt. } Fe^{3+} + NO_2^- + H_2O \quad [4]$$

' The abbreviations used in this chapter include :
PN^+ for the oxidized pyridine nucleotide carrier,
PNH for the reduced pyridine nucleotide carrier,
FAD and FAD_2 for the oxidized and reduced forms of flavin adenine dinucleotide,
cyt. Fe^{3+} and cyt. Fe^{2+} for the oxidized and reduced forms of cytochrome c,
[H] for unspecified biological reducing power.

The molybdenum-containing enzyme of the nitrate reductase system competes with the cytochrome oxidase of the ordinary oxygen-consuming system for electrons from cytochrome-c. Therefore, oxygen and nitrate compete with each other for electrons generated during metabolism of those organisms capable of using both nitrate and oxygen as acceptors (Campbell and Lees, 1967). In these organisms oxygen competes more successfully. A control mechanism exists by which the activity of the dissimilative enzyme system is repressed by oxygen (Woldendorp, 1963; Delwiche and Bryan, 1976).

Dissimilatory nitrate reductases are reported to be membrane-bound with the exception of *Spirillum itersonii*, where the enzyme is found in extracts and disrupted cells (Delwiche and Bryan, 1976). In addition, enzyme preparations made from bacterial cells catalyze nitrate to NO, NO to N_2O, and N_2O to N_2 or the reduction of nitrite to NO, N_2O and N_2. The responsible enzymes are termed the nitrite, nitric oxide and nitrous oxide reductases, respectively (Payne *et al.*, 1971; Alexander, 1977).

2.3 Intermediates of Nitrate Reduction

Several major pathways of nitrate reduction have been proposed and different intermediates detected, but their obligatory presence in the process is still tenuous and remains the subject of some controversy after years of study

by numerous workers (Alexander, 1977; Ardakani *et al.*, 1975; Campbell and Lees, 1968; Dewiche and Bryan, 1976). A general scheme representing the major nitrate reduction pathways involves the reduction of nitrate to nitrite, and then, in the denitrification sequence, nitrite is transformed to nitric oxide (NO), which in turn is converted to N_2 with nitrous oxide as an intermediate. On the other hand, the mechanism of nitrite reduction to ammonium remains uncertain. In particular, the evidence indicating hydroxylamine (NH_2OH) as an intermediate remains tenuous (Alexander, 1977).

According to Delwiche and Bryan (1976), if two electrons are added to nitrite, the resultant compound, nitroxyl (HNO) readily dimerizes and yields hyponitrite . Hyponitrite decomposes rapidly in acid solutions and at measurable rates in neutral solutions, yielding nitrous oxide (N_2O) and water. Campbell and Lees (1968) proposed that NO_2^- is reduced to nitrohydroxylamine (NO_2-NH-OH), which in turn decomposes to nitrous acid (HNO_2) and hydroxylamine (NH_2-OH). The hydroxylamine could be either reduced directly to ammonia (NH_3) or reduced stepwise to nitrous oxide (N_2O) and finally to nitrogen (N_2). Both of the above schemes are speculative and only future research can decide whether or not they are correct.

It has been suggested that in enzymatic nitrite reduction the intermediates remain bound to the enzyme surface until ammonia or N_2 is released. This could explain

how living cells can succeed in stoichiometrically converting nitrite into ammonia or gaseous nitrogen via different intermediates, such as nitrohydroxylamine or nitrous oxide (Woldendorp, 1968; Campbell and Lees, 1968). If the latter proves to be true, it will help to explain many of the conflicting data on the mechanism of nitrate reduction.

2.4 Magnitude of the Assimilatory Pathway

Over the years, contradictory results have been reported for the magnitude of the assimilatory NO_3^--N reduction process in soils under anaerobic conditions.

Basically the differences in the assimilatory and dissimilatory pathways reflect the physiological role of each process. The assimilatory pathway supplies reduced nitrogen for biosynthesis, and as such reduces only as much NO_3^--N as is needed for growth. Therefore, the main regulator is NH_4^+-N and the process is insensitive to oxygen (Payne, 1973). Though not regulated by O_2, assimilatory reduction would be expected only in aerobic habitats, since the repressor, NH_4^+-N , is generally high in anaerobic habitats owing to the absence of nitrification (Tiedje *et al.*,1981). In contrast, the dissimilatory pathway functions as electron acceptor, which allows greater energy conservation and thus more efficient growth (Thauer *et al.*, 1977). Because O_2 is the preferred electron acceptor, this pathway is mainly

regulated by O_2 , and is not inhibited by NH_4^+-N (Burish and Patrick, 1978; Caskey and Tiedje, 1979).

In agreement with Thauer *et al.* (1977), Focht (1974) considers that under anoxic conditions, assimilatory losses are insignificant in light of thermodynamic considerations. He cites the following reasons. More energy is required for the reduction of NO_3^--N to NH_4^+-N than to N_2 . The incorporation of one atom of nitrogen into bacterial biomass requires approximate incorporation of 7 atoms of carbon, such that the minimal C/N ratio has to be 7/1. Besides, the most efficient manner of generating the energy required for biosynthesis is through oxidative phosphorylation, which is best achieved by coupling the oxidation of carbonaceous substrate with nitrate (in lieu of O_2) as the electron acceptor. Assimilation of nitrogen by fermentative non denitrifying organisms would be much less efficient and would involve considerably more carbonaceous substrate because of incomplete oxidation.

Although an accumulation of NH_4^+-N during denitrification in soils has often been observed, experiments with labelled nitrate have indicated that the ammonium pool has not contained appreciable amounts of label (Broadbent, F.E., 1952; Nommick, H, 1956 ; Wijler, J. and Delwiche, C.C, 1954). Woldendorp (1963), in an investigation to determine to what extent *Bacillus licheniformis* was capable of reducing NO_3^--N to NH_4^+-N under conditions prevailing during denitrification in the rhizosphere found

that approximately 1.5% of the added NO_3^--N was reduced to NH_4^+-N. He concluded that the greater part of the NH_4^+-N was derived from deamination reactions. Also, Sacks and Barker (1952), in a defined medium, showed that 98% of the added NO_3^--N (717 μg-N/ml) was converted to N and the remainder left intact with growing cells of *Pseudomonas denitrificans*. All of the NH_4^+-N and cellular nitrogen appeared to have been derived from glutamic acid, the only other nitrogen source present.

More recent work with ^{15}N labelled NO_3^--N has shown that between 0.3 to 0.6 μg N $g^{-1}day^{-1}$ were reduced to NH_4^+-N in an anaerobic soil (Tiedje *et al.*, 1981). Fermentative soil anaerobes such as *Clostridia* have been found responsible for the reaction (Caskey and Tiedje, 1979). According to Tiedje *et al.* (1981), this reduction is a respiratory rather than a growth-linked process, which means that NH_4^+-N should accumulate and be excreted from the cell. A fraction could pool with NH_4^+-N from other sources with only a portion incorporated into biomass. Thus in ^{15}N labelled studies, some ^{15}N from NO_3^--N could be expected to be converted to organic form, but only an amount proportional to its contribution to the total NH_4^+-N pool.

Research with labelled NO_3^--N reveals that the formation of labelled NH_4^+-N , is enhanced by glucose (Buresh and Patrick, 1978; Caskey and Tiedje, 1979). Stanford *et al.* (1975,a) found that when the C/N ratio of added glucose and nitrate was 10 in a silt loam soil, approximately 19% of the

added $^{15}NO_3^-$-N was present in the NH_4^+-N pool and 18% in the soil organic matter, after 24 hours of anaerobic incubation. In an experiment with anaerobic slurries treated with $^{15}NO_3^-$-N and glucose-C (C/N = 5), Tiedje *et al.* (1981), observed two distinct phases for NO_3^--N reduction to NH_4^+-N and denitrification. During the initial part of the growth phase (35 hours), the $^{15}NH_4$-N concentration was lowered, whereas during the late phase (48 hrs), the production of NH_4^+-N from NO_3^--N and NO_2^--N reduction was excessive and accounted for most of the NH_4^+-N production at this stage. The latter workers consider that this high activity of reduction to NH_4^+-N is associated with growth, the bacteria responsible possibly being different from the denitrifiers and requiring more reduced conditions before the excessive growth. Focht (1978) considers that NO_3^--N will not be assimilated as long as an exogenous energy source is absent or if the addition of an exogenous energy source does not exceed the stoichiometric C/N ratio required for the completion of the reaction to carbon dioxide and dinitrogen. The following equation can be used to calculate the amount of available carbon required for microbial reduction to N_2 :

$$5(CH_2O) + 4NO_3^- + 4H^+ \longrightarrow 5\,CO_2 + 2N_2 + 7H_2O \qquad [10]$$

Thus, 1 g of available carbon is required for the production of 0.93 g of N as N_2 and therefore the C/N ratio is 1.07 (Burford and Bremmer, 1975). C/N ratios higher than 1.07 may represent a significant influence in cell growth and population shifts, hence affecting the NH_4^+-N production

and subsequent immobilization, in a way that has not been determined.

From the previous review it can be concluded that NO_3^--N reduction to NH_4^+-N and further immobilization can occur in soils under anaerobic conditions. This process is affected by the amount of carbon available for microbial growth in relation to the NO_3^--N present in the system (C/N ratio), and it is greatly favoured by increased reducing conditions in the system. The exact mechanisms by which these factors influence the process are yet to be determined.

2.5 Microbiology

Arable fields contain an abundance of denitrifying microorganisms, and counts in excess of a million per gram are not uncommon in field soil. The population is typically larger in the immediate vicinity of plant roots. This , however, only demonstrates a potential, rather than actual, activity for rapid nitrogen volatilization. (Alexander, 1977).

Most of the bacteria responsible for reduction of NO_3^--N to gaseous forms of nitrogen are facultative; under aerobic conditions they can use oxygen as an electron acceptor, and under anaerobic conditions they can use NO_3^--N and NO_2^--N as final electron acceptors, provided that an oxidable substrate is present in the medium (Delwiche and Bryan, 1976).

Most denitrifiers are heterotrophic, but several chemoautotrophs are capable of reducing nitrate to molecular nitrogen. *Paracoccus denitrificans*, a facultative autotroph, develops in air or anaerobically, with either organic compounds or H_2 as sources of energy, and O_2 or nitrate as electron acceptors. *Thiobacillus denitrificans* is a sulfur-oxidizing chemoautotroph that differs from other *Thiobacilli* by its ability to proliferate anaerobically providing nitrate is available. For *T. denitrificans*, the energy source in these circumstances is sulfide, elemental sulfur, or thiosulfate, all of which are oxidized to sulfate. The nitrate is converted mainly to N_2 , but NO and N_2O are also sometimes evolved. (Alexander, 1977).

Most denitrifiers reduced nitrate all the way to N_2, using NO_3^-, NO_2^- , NO or N_2O as electron acceptors for proliferation. Some bacteria, however, carry out incomplete reductions. *Corynebacterium nephredii*, for example, reduces only NO_3^- , NO_2^- , and NO , but the compound at the end of the sequence with this bacterium is N_2O rather than N_2 (Alexander, 1977). Others lack nitrate reductase and are thus NO_2^--dependent . Some lack the ability to reduce NO_2^- to N_2O, but have the ability to reduce N_2O.

Two species , *Azospirillum brasilense* and *Rhodopseudomonas sphaeroides* , have the ability to denitrify as well as to fix N_2 , but the ecological significance of such abilities is not clear yet (Knowles,1981). According to Alexander (1977), the active species are largely limited

to the genera *Pseudemonas*, *Bacillus* and *Paracoccus*. In a recent review, Knowles (1981), listed 20 genera, indicating their main characteristics regarding their ability to denitrify. Perusal of Bergey's Manual (1974) indicates that denitrifying bacteria are widely distributed taxonomically, suggesting that the capacity of bacteria to denitrify is not confined to a few genera.

2.6 Factors Affecting Denitrification

2.6.1 Nitrate Concentration.

Several reports have indicated that nitrate concentration can influence dissimilatory nitrate reduction and the relative proportions of intermediate compounds as well. (Delwiche and Bryan, 1976; Malhi , 1978; Woldendorp, 1963; Yamane, 1969). Alexander (1977) states that nitrous oxide release in soil or in culture is conditioned by nitrate concentration and the relative proportion of the gas is greatest at high nitrate levels. A later study by Cho *et al*. (1978), confirmed that accumulation of N_2O was dependent upon the NO_3^--N concentration; with higher concentrations of NO_3^--N favoring accumulation of N_2O, thus reducing the formation of N_2. He attributed this finding primarily to the competitive nature of NO_3^--N and N_2O as electron acceptors under constant microbial activity. Nevertheless, recent results contradict these conclutions. Blackmer and Bremmer

(1979) suggested that nitrate may have two effects on this process; one being that it stimulates N_2O reduction to N_2, and the other being that it can inhibit the reaction. Gaskell *et al.* (1981) comparing the effects of NO_3^-, NO_2^- and mixtures of NO_3^- and NO_2^- on reduction of N_2O to N_2 , found that in some instances, NO_3^-, NO_2^- and NO first inhibited and then stimulated the reduction of N_2O to N_2 by soil microorganisms. They also found that NO_3^- alone inhibits N_2O reduction, but they did not confirm the suggestion by Firestone *et al.* (1979), that this inhibitory effect was due to nitrite formed through microbial reduction of nitrate. It can be concluded that nitrate concentration is an important factor affecting intermediates of denitrification but further studies are needed to clarify the mechanisms involved.

2.6.2 Temperature

Several optimum temperature values for denitrification in soil have been presented: about 60°C by Bremner and Shaw (1958), 65°C by Nommick (1956), 30°C by Bollag *et al.* (1970), 25°C by Alexander (1977) and 40°C for soils of the province of Alberta by Malhi (1978).

The lower temperature limits of denitrification have been set: at 2°C by Bremmer and Shaw (1958), at 3°C by Nommick (1956), at 5°C by Bailey and Beauchamp (1973 c), at 10°C by Bollag *et al.* (1970), at 2 to 5°C by Stanford *et al.* (1975), and at -4 to -5°C for soils of the Province of

Alberta by Malhi (1978).

The upper temperature limits have been set at 70°C by Bremner and Shaw (1958), at 85°C by Nommick (1956) and at 60 to 65°C by Malhi (1978).

Temperature affects the relative proportion of intermediates formed during denitrification. Bailey and Beauchamp (1973c) reported that a partial inhibition of the reduction of N_2O to N_2 occurs at 8°C, 10°C, and 15°C, resulting in accumulation of N_2O and NO_2^--N .

Later, Bailey (1976) reported that decreasing the temperature from 30 to 6-8°C, resulted in an increase in NO production; this was the principal gas produced at 6-8°C. Nitrous oxide (N_2O) production, like NO, increased as the temperature decreased from 30 to 10°C, where it was the principal gas produced. At 30 to 15°C and 6-8°C, N_2O production was secondary in quantity to N_2 and NO, respectively. The NO and N_2O released due to this temperature effect, may have an influence on O_3 in the upper atmosphere.

In Central Alberta, losses from soil N and fertilizer N have been reported to occur at the beginning of the spring when the soil is saturated with water from snowmelt (Nyborg and Leitch, 1979; Malhi, 1978). Malhi, using ^{15}N labelled fertilizer, found that N losses in early spring are almost exclusively through denitrification. The average monthly soil temperature at 10 cm (10 yr period October 1964 to September 1975) reported for the spring months ranges

between -3.8 and 10.0°C (Malhi and McGill, 1982). Therefore, a native denitrifying microflora adapted to these climatic conditions could have an important impact on the environment, if as reported earlier, the proportion of N_2O and NO released is increased at this temperature.

2.6.3 pH

The overall rate of production of gaseous products of denitrification is positively correlated with pH, having an optimum between 7.0 and 8.0 (Nommick, 1956; Van Cleemput and Patrick, 1974; Wigler and Delwiche, 1959; Woldendorp, 1968).

Many of the bacteria that bring about denitrification are sensitive to high hydrogen ion conentration, and hence various acid soils contain a sparse denitrification population (Alexander, 1977). Woldendorp (1968) found that below pH 6 denitrification was markedly retarded, with N_2O being the main product. Above pH 10 and below pH 4.5 the process was found to be completely suppressed.

Delwiche *et al*. (1976) reported that pH of soils influences both denitrification rates and the relative proportions of N_2, N_2O and NO. Alexander (1977) attributed these differences in gas composition associated with pH to an acid sensitivity of the N_2O reductase enzyme system.

2.6.4 Oxygen Level

It is now generally accepted that dissimilatory nitrate reduction in most organisms becomes dominant only under

anaerobic conditions (Alexander, 1977; Delwiche and Bryan, 1976). The quantity of dissolved oxygen is controlled by oxygen consumption (plants, microflora, microfauna), diffusion rate, partial pressure of oxygen in the soil atmosphere and temperature. The partial oxygen pressure is also influenced by other factors such as moisture level, texture and structure of the soil (Woldendorp, 1963). Although the mechanism by which oxygen affects nitrate is not well understood, denitrification has been reported to be controlled by oxygen repression of nitrogen reductase transcription (Woldendorp, 1963; Delwiche and Bryan, 1976; Cox and Payne, 1973; Knowles, 1981). Aerobically grown cells placed under anaerobic conditions exhibit a lag period before using of nitrate as an electron acceptor. No lag has been reported in the use of oxygen by anaerobically grown cells. After the metabolite repressor is removed, finite time is required, presumably to transcribe and translate the genes involved. In *Pseudenomas perfectomarinus*, the synthesis of the denitrifying enzymes begins within 40 minutes after derepression (Delwiche and Bryan, 1976). The reduction of NO_3^--N to NO_2^--N seems to be less sensitive to O_2 than are the later steps. Thus, as denitrification is repressed by increasing exposure to O_2, reduction of N_2O to N_2 is altered and an increasing proportion of N_2O is released (Knowles, 1981).

In experiments with *Pseudenomas denitrificans*, Sherman and MacRae (1957), observed that concentration of dissolved

oxygen above 0.2 ppm suppressed dissimilatory reduction of nitrate completely. Greenwood (1962), found similar results. On the basis of laboratory data, Woldendorp (1963) concluded that the low levels of oxygen required for denitrification will often occur in soils under normal agricultural practice.

2.6.5 Moisture Level

The effect of water on denitrification is attributed to its role in governing the diffusion of O_2 to sites of microbiological activity (Alexander, 1977). Furthermore, water can act as a solvent for nutrients needed by the denitrifying population, as well as control the diffusion rate of these nutrients between microsites of the soil environment.

Studies suggest that denitrification occurs at water contents above 60% of maximum water holding capacity (Bremmer and Shaw, 1958; Nommik, 1956) The latter found that at 100% saturation, denitrification was 9 times greater than at 80% saturation.

According to Alexander (1977), at moisture levels above 60% of the water-holding capacity, the rate and magnitude of denitrification are correlated directly with the moisture regimen.

2.6.6 Carbon Sources

In soil, the reducing agents used during denitrification are mainly supplied by organic compounds. Denitrifiers can utilize a wide array of carbohydrates, organic acids, or the organic compounds of complex culture media as carbon and energy sources during aerobic growth. Under anaerobic conditions, sequences requiring oxygenase activity may be suppressed and the substrate spectrum restricted for some organisms, i.e. *Pseudonomas stutzeri* uses valine, leucine, isoleucine and cysteine during aerobic growth but not under denitrifying conditions (Delwiche and Bryan, 1976).

In general, levels of native soil organic matter have been reported to be sufficient for the occurrence of denitrification. Bremner and Shaw (1958) found denitrification to be absent only when the quantity of soil organic matter was less than 1%, and observed that readily decomposable materials such cellulose rather than lignin , wheat straw rather than water-extracted wheat straw, strongly stimulated denitrification.

Burford and Bremner (1975), reported that denitrification capacities of the soils they studied were significantly correlated (r=0.77) with total organic carbon and very highly correlated (r=0.99) with water-soluble carbon or mineralizable carbon (evolved CO_2-C). This work strengthens the idea that denitrification in soils under anaerobic conditions is controlled largely by the supply of

readily decomposable organic matter and that analysis of soils for mineralizable carbon or water-soluble organic carbon provides a good index of their potential for denitrification.

Stanford *et al.* (1975) determined denitrification rates under near anaerobic conditions in 30 soils of diverse origin that differed widely in pH , organic matter content and other characteristics. They found that correlations of the apparent first-order rate constant (k), denoting the fractional loss of NO_3-N $hour^{-1}$, with total soil organic C and with soil "glucose-C" were highly significant. It is therefore clear that the carbon supply plays a major role in denitrification. Nevertheless, no information has been gathered regarding the dynamic changes of the carbonaceous pool while NO_3-N losses are taking place.

2.6.7 Influence of Plants

Denitrification rates are more rapid in a system containing plants than in a fallowed one (Bailey, 1976; Alexander, 1977) ; in addition to creating anaerobic microsites in the rhizosphere, plants promote denitrification by the excretion of hydrogen donors or a combination of both. The number of denitrifying organisms has been found to be higher in the rhizosphere than in non-rhizosphere soils. Moreover, a continuous supply of nitrate is assumed after the application of nitrate-containing fertilizer, as a consequence of uptake by

roots, thus causing a constant flow of this soluble compound through the rhizosphere. Consequently, rhizosphere soils constitute an excellent environment for the occurrence of denitrification. (Woldendorp, 1963, 1968; Bayley, 1976; Alexander, 1977).

2.7 Kinetics of Denitrification

Considering that denitrification takes place in a biological system, nitrate may be consumed at a rate represented by a Michaelis-Menten expression(fig.1). The Km value may be termed an affinity constant between nitrate and nitrate reductase, v is the observed rate of reaction, Vmax is the maximum rate of reaction that the system can achieve, and [S] is the nitrate concentration. Figure 1 can be represented by the following equation:

$$v=(Vmax[S])/(Km + [S]) \qquad [11]$$

When NO_3^--N is very limiting, the system follows a first order rate of loss. At a greater NO_3^--N supply, the degree of saturation of nitrate reductase increases and at saturation the rate of loss becomes independent of additional nitrate. The same argument can be applied to organic carbon (Cleland, 1970; McGill,(1977); Requa and Shroeder, 1973).

In the case of two substrates, the following expressions may be used for denitrification:

$$v= (Vmax[C][N])/((C + Kc)(N + Kn)) \qquad [12]$$

where [C] or [N] correspond to carbon or nitrogen

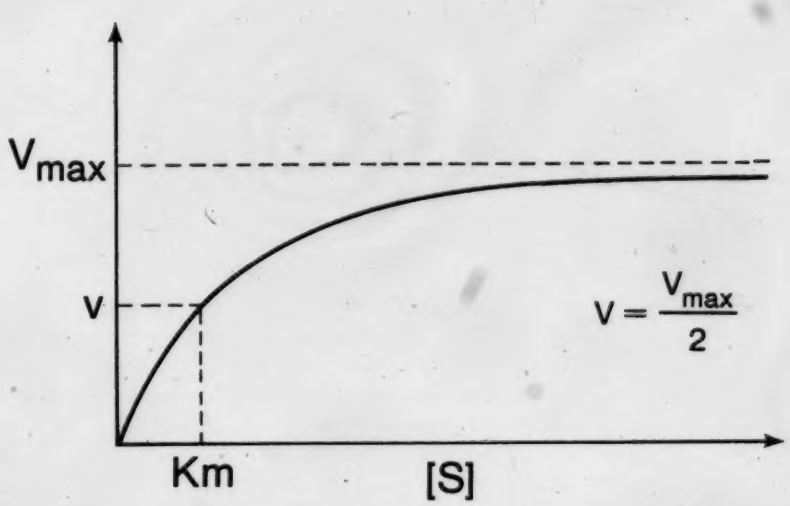

Figure 1. Representation of Michaelis-Menten Kinetics

concentration, Kc or Kn correspond to half saturation constants, and v and Vmax as already defined (Bray and White, 1966).

Besides Michaelis-Menten expressions, which suggest equilibrium reactions, there are three reaction order expressions that have been used to represent denitrification rate: zero order, first order and second order.

The rate of a zero order reaction can be represented by the expression:

$$dN = R\,dt \qquad [13]$$

where dN represents the change in NO_3^--N content , R represents the rate of NO_3^--N loss and dt represents the time span considered. This is the simplest system and is accurate at reasonably large NO_3^--N concentrations.

The rate of a first order reaction can be represented by the expression:

$$dN = -kN\,dt \qquad [14]$$

which integrates to

$$Nt = No\,e^{-kt} \qquad [15]$$

where Nt is the amount of nitrate present at a given time t, No is the nitrate present at time zero, e is the base of natural logarithms and k is the first order rate constant. A first order approach can be used if nitrate is low.

The second order approach considers two substrates involved in the reaction. Use of the second order expression assumes that C is either completely oxidized to CO_2-C and/or is used for biomass production on a weight basis, concurrent with complete reduction of NO_3^--N to N_2 . Considering that 1 atom of nitrate will consume 5 electrons during its reduction to N_2 , it should take 4.8 atoms of nitrate to accept 24 electrons generated during oxidation of one atom of a simple sugar ($C_6H_{12}O_6$) to CO_2-C and H_2O. Thus, one atom of NO_3^--N should accept the electrons from 1.25 C atoms. This produces a C:N ratio of 1.07 (wt:wt) (McGill, 1977). Most data available, though, indicate that the C:N ratio is closer to 2 (Bowman and Focht, 1974). Based upon complete oxidation of CH_2O to CO_2 , equation [10] (page 10) should represent the system. Assuming the difference to be due to biomass production within the microbial pool, the following equation may be more representative:

$9.33(CH_2O) + 4NO_3^-N + 4H^+ \longrightarrow$

$\longrightarrow 4.33(biomass\text{-}CH_2O) + 5CO_2 + 2N_2 + 7H_2O$ [16]

On a wt. basis, the equation becomes:

$112C + 56N \longrightarrow 52(biomass\text{-}C) + 60\ CO_2\text{-}C + 56N$ [17]

which can be simplified to:

$2Ao + Bo \longrightarrow Products$ [18]

where Ao and Bo represent the initial concentration of C and N, respectively, and 2 molecules of reactant A are required per unit of reactant B. If x represents the change in concentration from B, and Bt and At represent the respective concentration after time interval t, then

$Bo - x = Bt$ [19]

$Ao - 2x = At$ [20]

and $dx/dt = k(Ao - 2x)(Bo - x)$ [21]

Which integrates to (Latham, 1969):

$(Ao - 2Bo)kt = \ln((Bo/Ao)\ ((Ao - 2x)/(Bo - x)))$ [22]

where k is defined as the second order rate constant, which can be determined experimentally by plotting $\ln((Bo/Ao)(At/Bt))$ on the ordinate against t on the abscissa. The slope of the line equals $(Ao - 2Bo)k$. Because $(Ao - 2Bo)$ is a constant obtained from the original concentration, k can be readily calculated. The values of At and Bt can be calculated from the integrated form of the equation:

$$(Ao - 2Bo)\, kt = \ln ((Bo/Ao)(At/Bt)) \quad [23]$$

$$e^{(Ao - 2Bo)\, kt} = (Bo/Ao)(At/Bt) \quad [24]$$

$$(Ao/Bo)\, e^{(Ao - 2Bo)\, kt} = At/Bt = y \quad [25]$$

and also, $(Ao - At)/2 = Bo - Bt$ [26]

therefore, $Bt = Bo - (Ao - At)/2$ [27]

then substituting in equation [25]

$$At/(Bo - (Ao/2) + (At/2)) = y \quad [28]$$

and clearing At in equation [28]

$$At = (y\, Bo) - ((Ao\, y)/2) + ((At\, y)/2) \quad [29]$$

$$At - ((At\, y)/2) = y\, (Bo - (Ao/2)) \quad [30]$$

$$At\, (1 - y/2) = y\, (Bo - (Ao/2)) \quad [31]$$

$$At = y\, (Bo - (Ao/2))/(1 - (y/2)) \quad [32]$$

$$At = y\, (2Bo - Ao)/(2 - y) \quad [33]$$

now, replacing y by equation [25]

$$At = \frac{(2Bo - Ao)(Ao/Bo)\, e^{(Ao - 2Bo)\, kt}}{2 - (Ao/Bo)\, e^{(Ao - 2Bo)\, kt}} \quad [34]$$

Theoretically, therefore, from the chemical kinetic standpoint, denitrification could be treated as a second order system.

Because the denitrification rate can be represented in several ways if the appropiate conditions are met, reports in the literature on this matter appear confusing. The

reaction order used to describe denitrification should be selected in a way that reflects the real system as closely as possible, taking into account the relative amounts of C and NO_3-N involved.

When high concentrations of NO_3-N and carbon are present, the reaction will be independent of either substrate concentration, and the system will have the maximum rate of reaction. In such cases, the reaction rate can be described using zero order kinetics, as found by Doner *et al.* (1974) in soil column studies. Smith and Beauchamp (1976) and Reddy *et al.* (1978) reported zero order kinetics during denitrification in the presence of an added energy source.

Nevertheless, rates of denitrification that approximate first order kinetics or two-substrate Michaelis-Menten kinetics have been reported (Bowman and Focht, 1974; Reddy *et al.*, 1980; Smith and Beauchamp, 1976; Stanford *et al.*, 1975). Bowman and Focht, while studying the influence of glucose and nitrate concentrations upon denitrification rates in a sandy soils found that denitrification rates were dependent on NO_3-N concentrations and glucose concentration; the denitrification rates were substrate-dependent at lower concentrations approximating first order kinetics and gradually diminished at higher concentrations to become independent of either substrate concentration (zero order kinetics). Furthermore, Stanford *et al.*, (1975) while studying sandy soils with very low contents of total or

extractable glucose-C found relatively poor fits for either first or zero order kinetics. They postulated that these soils could not sustain a steady rate denitrification for more than a few days because their sources of carbon soon were depleted.

On the other hand, Yamane (1969), reported decreasing NO_3^--N reduction rates as nitrate concentrations increased from 200, through 400, 800, and 1600 to 3200 $\mu g\ g^{-1}$ of NO_3^--N. Also, Bowman and Focht (1974) found that high glucose-C concentrations (1.8%) appeared to inhibit denitrification during anaerobic incubation.

From the previous review, it seems apparent that the rate of denitrification will fit several different kinetic treatments depending on the relative amounts of NO_3^--N and carbon present in the system, at a given time. Also, it is important to keep in mind that any of the simple treatments represented here will hold only for a short time. If one of the substrates is exhausted, the reaction will stop. The best approach therefore considers both substrate concentrations: either two substrate Michaelis-Menten kinetics, or the second order system.

2.8 Summary

It can be concluded that denitrification is controlled by several independent factors, many of which undergo seasonal and even shorter-term fluctuations and which

interact with each other. Thus, the complexity of the denitrification process and its control mechanisms make it extremely difficult to predict, for any particular environment, the overall denitrification rate and the relative proportions of products, unless the controls are quantified. Further research is needed to clarify aspects of denitrification such as, enzymic mechanism, intermediates and microbiology and their interrelationship with factors such as temperature, pH, and substrate concentrations. If such understanding is achieved, the ability to modify denitrification by appropriate soil management practices will increase.

3. Materials and Methods

3.1 Soils

Samples of Ap horizons were collected from two soils during the autumn of 1977. The soils were: a Black Chernozemic, Malmo SiCL, and a Gray Luvisolic, Breton L, located at the University of Alberta Ellerslie farm (NE-24-51-25-W4) and at the University of Alberta Breton Plots (NE-25-47-4-W5), respectively. The Malmo soil was cropped with alfalfa (*Medicago sativa*), unless otherwise specified. The Breton soil was under stubble. Table A of the appendix presents the general characteristics of each soil.

3.2 Sample Treatment

Soil samples taken from the field were air-dried and ground to pass a 2 mm sieve. Bulk samples were stored dry in the dark at room temperature until used for incubation studies.

3.3 Incubation Procedure

Each sample consisted of 100 g of air-dried soil placed in a 150-ml Erlenmeyer flask. Deionized water was used to saturate the soil; saturation being previously determined by recording the moisture needed to produce a glistening sheen on stirred samples. The incubation flasks were closed with a rubber stopper, therefore moisture losses were considered

insignificant during the incubation period. Incubations were in the dark at 28 C. In some experiments, prior to addition of amendments, the soil samples were pre-incubated for seven days at field capacity[3] to avoid the flush of activity following moistening of an air dried soil sample.

3.4 Amendments

Amendments (NO_3^--N , NO_2^--N and glucose-C) were applied dropwise in solution and mixed into the soil at time zero of all incubations. The sources of NO_3^--N and NO_2^--N were reagent grade $Ca(NO_3)_2$ $4H_2O$ and KNO_2 respectively.

Sampling during incubations was destructive; i.e. incubation flasks were not subsampled.

3.5 Analytical Procedures

3.5.1 Moisture Content

Soil samples were dried at 105 C for 24 h. with moisture loss expressed as percent of oven dry soil weight.

3.5.2 Denitrifying Population Size

The most probable number method, utilizing NO_2^--N broth (Voltz, 1977b) was used to estimate number of denitrifiers. Absence of NO_2^- was taken as indicating presence of denitrifiers.

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[3] % moisture at field capacity was defined as the % water retained at 33 kPa moisture tension. Values are expressed as percentage oven dry weight of soils.

3.5.3 Water soluble organic carbon

The water soluble organic carbon(WSOC) content was measured by shaking 10 g of soil in 100 ml of glass distilled deionized water in a polyethylene centrifuge bottle for one hour followed by centrifugation at 10000 x g, at 4 C for 30 minutes. The supernatant was filtered with suction through a 47 mm dia 0.2 μm metricel membrane filter previously washed with 100 ml deionized water. The filtrates were stored at -10 C until analysis was performed.

During the completion of this study, three methods to determine the WSOC content were used.

3.5.3.1 Persulfate Oxidation

The method used was taken from Hu *et al*. (1972). In this method a 20 ml aliquot of soil extract was placed in a 250 ml wide mouth Erlenmeyer flask (Corning 5100). Immediately , 1 ml of concentrated H_2SO_4 , 1 ml of saturated Ag_2SO_4 solution and 4 g of $K_2S_2O_8$ were added. A vial containing 1.8 ml of 1 N NaOH solution was placed into the 250 ml flask containing the sample-reagent mixture. The flask was stoppered and the stopper taped onto the flask which was placed in an oven at 80°C for 3 hours for oxidation of the C and collection of CO_2-C. The flasks were removed from the oven, allowed to cool, and the vial contents titrated to the phenolphthalein end point with 0.005 N HCl following addition of 1 ml of 1 M of $BaCl_2$.

3.5.3.2 Dichromate Oxidation

Extracts (10 ml) were treated with 15.0 ml of 0.267 N solution of $K_2Cr_2O_7$ in a 125 ml Erlenmeyer flask fitted with a reflux condenser, and the mixture boiled for 30 minutes (Mebius, 1960). Residual dichromate in the cooled digest was determined by titration on with 0.03 N Mohr salt ($(NH_4)_2SO_4FeSO_4 6H_2O$), with the indicator prepared by dissolving 200 mg of N-phenylanthranilic acid in 0.2% Na_2CO_3 solution.

3.5.3.3 Dry Combustion

In the final set of experiments, the WSOC content was determined using the Beckman Total Organic Carbon Analyzer model 915-B. Total C content and the inorganic C were both determined, with the organic C content taken as the difference (difference method).

3.5.4 Mineral and Total Nitrogen

Mineral N was extracted by shaking for 1 hour 10 g of soil with 100 ml of 2 M KCl-PMA solution followed by filtration of the resulting suspension (Douglas and Bremner, 1970). Determinations of NO_3^--N , NO_2^--N and NH_4^+-N were performed using steam distillation (Bremner, 1965a). For total nitrogen soil samples (0.1 g) were pre-treated using $KMnO_4$ solution to oxidize NO_2^- to NO_3^- followed by powdered Fe to reduce NO_3^- to NH_4^+ . Samples were then digested according to the normal Kjeldahl procedure, but using 7 ml of $H_2SO_4/K_2SO_4/CuSO_4$ solution (McKeague, 1978). Ammonium

produced was measured using steam distillation and collection in 4% boric acid (McKeague, 1978).

3.5.5 Evolution of CO2-C

Evolution of CO_2-C during the incubation period was measured in quart jars using the NaOH collection method outlined by Middleboe *et al.* (1976).

3.5.6 Total Soil Organic Carbon

Total soil organic C was determined by dry combustion in the Leco furnace (Tabatabai and Bremner, 1970).

3.5.7 Exchangeable Cations

Exchangeable Na, K, Ca and Mg were determined by atomic absorption (McKeague, 1978).

3.5.8 pH

Soil pH was measured with a pH meter using a glass electrode in a suspension of 1:2.5 , soil:water ratio.

3.5.9 Total Phosphorus

Total phosphorus was determined using the digestion procedure outlined by Parkinson and Allen (1975) and measured using the molybdenum blue method (McKeague, 1978).

3.5.10 Total Sulfur

Total sulfur was determined using the alkaline oxidation method as outlined by Tabatabai and Bremner (1970).

4. Effect of Pretreatment on Water Soluble Organic Carbon of Two Soils

4.1 Introduction

Much of the total organic carbon of soil is highly resistant to decomposition. The short residence time of the water soluble organic fraction indicates it is a more dynamic component which is particularly susceptible to decomposition (McGill,1978). In a study related to revegetation of the Oil Sand Area in Alberta, a close relationship between respiration rate and soluble carbon supply was found in several soils. In every soil, CO_2-C evolution rates dropped off quickly once soluble carbon content dropped off, suggesting that for most practical purposes the soluble carbon pool is the immediate carbon source for soil microbes (McGill,1978).

Hu and Gilmour (1972) reported that the WSOC was a good index of decomposition and humification. They found that the levels of WSOC were consistently higher in the less decomposed materials and decreased with increasing decomposition and humification.

Burford and Bremner(1975) reported that the water soluble organic carbon content in soils provided a good index of their capacity for denitrification.

Reports in the literature indicate an important role for WSOC, but they do not clearly demonstrate the controls and mechanisms supplying carbon to the soil solution. The

objectives of this research were: i)to determine the WSOC content present in air-dried samples and pre-incubated samples of the Malmo and Breton soils; ii) to establish the most precise method to determined the WSOC content of the soil extracts; iii) to determine the effect of increased volumes of extractant per g of soil on the recovery of WSOC ; iv) and to determine the WSOC content of field moist samples and the relative proportion of C compounds of proteinaceous nature present in the WSOC pool.

4.2 Materials and Methods

Determinations of the WSOC content were performed on extracts obtained from air-dried samples, pre-incubated samples, and fresh moist samples taken from the field. Samples were replicated three times each. The extracts were obtained by shaking increasing volumes of water (extractant) with a constant mass of soil.

Analyses of WSOC were performed with the Total Organic Carbon Analyser Model 915-B, and two different methods were studied.

4.2.1 Difference Method

The extracts were analysed in the total carbon channel and in the inorganic C channel of the analyser and the organic carbon content determined by difference.

4.2.2 Acid-sparge Method

The extracts were acidified by adding one drop of concentrated HCl(10 M) and the samples were set in the sparge manifold and sparged using CO_2-free air to remove inorganic carbon. The organic carbon of the sample was then determined in the total carbon channel.

4.2.3 Ninhydrin Reactive N

The ninhydrin reactive N was performed in the extracts of the fresh field samples using the method developed by Moore and Stein(1954).

4.3 Results

4.3.1 Air dried Samples

Extracts of air-dried samples were analysed by using the Difference Method and the Acid-sparge Method in the Beckman 915-B analyser. Different water volume to soil mass ratios were used in both cases for each soil. Results of WSOC contents obtained with the Acid-sparge method were smaller than those obtained with the Difference Method, with two exceptions in the Breton soil(Appendix, Table B). The latter was attributed to natural variability within soil samples, since the relative amounts of WSOC in the Breton soil were very small.

Differences in results using these two methods could be attributed to the presence of volatile water soluble organic

compounds in the extracts, which would be lost in the process of acid sparging. Use of the Difference Method has been recommended for samples where the volatile organic carbon (VOC) content may be significant and includes various petroleum, petrochemical, and sewage waste waters. In these applications, the Total organic carbon levels are sufficiently high so that the difference technique (Total C - Inorganic C = Total organic C), is the preferred method (anon.,1979).

As a consequence, the Difference Method was adopted for determinations of the WSOC contents of the soils in the subsequent studies.

For air dried Malmo soil samples, WSOC contents varied from 423 up to 2126 $\mu g\ g^{-1}$, for the water volume to soil ratios of 2 and 100, respectively (Fig. 2). These results suggested that the greater the volume of extractant used per gram of soil, the greater the amount of WSOC content brought into solution.

The same trend was found in air dried samples of the Breton soil, but the magnitudes of extracted carbon at the different water to soil ratios were approximately one half of the Malmo soil extracts. The difference in WSOC contents of the two soils was significant at the 1% level as judged by a t test.

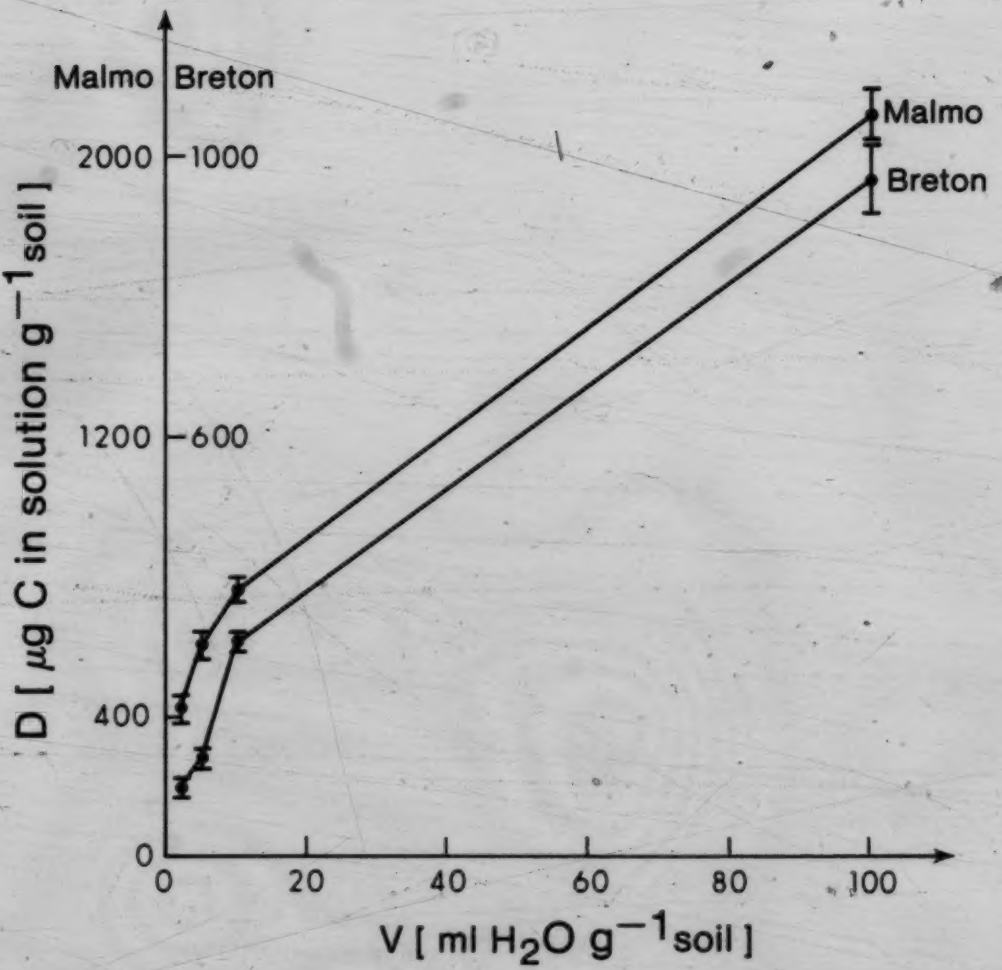

Figure 2. WSOC contents (D) in air-dried samples of the Malmo and Breton soils, extracted with different volumes of water (V). Bars indicate standard deviation.

4.3.2 Pre-incubated samples

For both soils with pre-incubated samples higher amounts of WSOC were extracted with increasing water to soil ratios (Tables 1 and 2). Amounts of WSOC extracted from the pre-incubated soils were, however, smaller than those extracted from air-dried samples.

The WSOC contents of pre-incubated Malmo samples were approximately one half of those of air-dried samples, at a given extraction ratio. In Breton soil samples the WSOC was about 1/3 to 1/4 as large in pre-incubated samples as in air-dried samples.

These different WSOC contents obtained for air-dried samples and pre-incubated samples can be attributed to microbial uptake of WSOC once the soil is incubated, followed by either conversion to biomass or release as CO_2-C during microbial metabolism.

The difference in WSOC contents of the two soils was significant at the 1% level as judged by the t test.

4.3.3 Field Moist Samples

WSOC contents of field moist samples were measured at two different volumes to soil weight ratios, to determine if a greater volume of extractant affected the magnitude of extracted carbon, as found in the previous experiments.

Samples under different management conditions, such as fallow, barley stubble, sod and hay were studied in the Malmo soil. Samples under stubble were studied in the Breton

Table 1. WSOC contents of extracts obtained by using different volumes of extractant to soil mass in pre-incubated samples of the Malmo soil (by the difference Method).

Volume of extractant/soil mass	WSOC
ml g^{-1}	$\mu g\ g^{-1}$
6	351 ± 4
11	371 ± 10
96	1100 ± 5
98	1092 ± 6

± indicates standard deviation.

Table 2. WSOC contents of extracts obtained by using different volumes of extractant to soil in pre-incubated samples of the Breton soil (by the difference Method).

Volume of extractant/soil mass	WSOC
ml g^{-1}	$\mu g\ g^{-1}$
5	52 ± 5
7	64 ± 6
10	69 ± 9
49	198 ± 14

± indicates standard deviation.

Table 3. WSOC contents of field moist samples of the Malmo and Breton soils extracted at two ratios of water to soil and the nynhydrin reactive-N contents of some of the extracts.

Soil	Management	Water Volume/Soil Mass	WSOC	Ninhydrin-reactive N
		ml g^{-1}	ug g^{-1}	leucine equivalent (ug g^{-1})
Malmo	Fallow	2	51 ± 9	
		5	88 ± 3	1.7
	Stubble	2	43 ± 2	
		5	73 ± 5	1.3
	Sod	2	56 ± 10	
		5	90 ± 5	2.8
	Hay	2	42 ± 4	
		5	69 ± 14	0.7
Breton	Stubble	2	17 ± 2	
		4	24 ± 3	1.6

± indicates standard deviation.

soil (Table 3).

In the Malmo soil, WSOC contents varied with management practices; the soil under sod had the highest amount of WSOC (90 $\mu g\ g^{-1}$) and the lowest WSOC content was in the one under hay (69 $\mu g\ g^{-1}$). On the other hand, the WSOC content of the stubbled Breton soil was about 1/3 of the stubble Malmo soil (24 $\mu g\ g^{-1}$). In both soils, increasing ratios of water to soil mass resulted in greater amounts of WSOC extracted per unit mass of soil. Variation of the WSOC contents between samples extracted with different volumes of extractant and under different management, however, were relatively small when compared to the WSOC contents extracted from air-dried samples and samples pre-incubated following air drying.

The ninhydrin reactive-N content of the extracts, indicated that the contents of amino acids and related compounds were small and varied with management practices (Table 3). In the Malmo soil the highest content was determined for the soil under sod and the lowest under hay, 2.8 and 0.7 $\mu g\ g^{-1}$ leucine equivalent, respectively. The Breton soil under stubble had a similar amounts of ninhydrin reactive N to that observed in the Malmo soil under stubble (1.5 and 1.3 $\mu g\ g^{-1}$ leucine equivalent, respectively).

4.4 Discussion

In general, data of the WSOC contents for air-dried samples, pre-incubated samples and field moist samples of the Malmo and Breton soils indicate that the soluble carbon

pool available in solution is different for these two soils and for a given soil appears to be weakly related to management conditions. Also, the data indicate that the total WSOC desorbed per mass of soil is related to the volume of extractant used.

4.4.1 Air-dried and Pre-incubated Samples

Because the measured WSOC contents of the soils were found to be related to the volume of extractant, it is convenient and useful to describe them in quantitative or mathematical terms. The Freundlich adsorption isotherm has been applied to the adsorption of nutrients and organics by soils and is, therefore, considered here. The Freundlich isotherm has a form that implies infinite ability to adsorb organic matter:

$$x/m = k\ Ce^{1/n} \qquad [1]$$

where x/m represent the amount of WSOC adsorbed per g of soil, k represent the adsorption constant having units of ml g^{-1}soil, n reflects the degree of nonlinearity of adsorption, and Ce represents the equilibrium solution concentration ($\mu g\ C\ ml^{-1}$) (McGill,1978). The two constants n and k embrace all factors affecting adsorption from solution: properties of the adsorbate, adsorbent, and the solvent, as well as the equilibria between the adsorbate-adsorbent, adsorbate-solvent, and solvent-adsorbent. The flexibility of the two constants(k

and n) allows easy curve fitting but does not guarantee accuracy if the data are extrapolated beyond the experimental points.

Because the Freundlich adsorption equation is an exponential function, it emplies that the energy of adsorption decreases logarithmically as the fraction of surface covered increases. Therefore, the adsorption of WSOC would be mainly a function of the total amount of WSOC and that adsorption can be increased almost indefinitely. The Freundlich equation can be derived theoretically by assuming that the decrease in energy of adsorption with increasing surface coverage is due to surface heterogeneity. Because the degree of heterogeneity is unknown in most adsorption investigations, the Freundlich equation is best treated as an empirical description of an actual adsorption process (Bohn *et al.*, 1979).

On the other hand, desorption can be experimentally determined by equilibrating increasing volumes (V) of extractant (water) with a constant weight of adsorber (soil). The concentration of desorbed material under study is determined quantitatively. At equilibrium, adsorption is directly proportional to solution concentration and conversely desorption is inversely proportional to solution concentration. At this point:

Adsorption ⟷ Desorption [2]

If adsorption is represented by:

$$x/m = fi\ Ce^{1/n} \quad [3]$$

then, if desorption is an inverse relation it can be represented by :

$$D = fj\ (1/Ce)^{n} \quad [4]$$

and has units of μg C/g of soil.

Considering that:

$$D = V \times Ce \quad [5]$$

then

$$1/Ce = V/D \quad [6]$$

therefore

$$D = k\ (V/D)^{n} \quad [7]$$

and

$$D = k\ V^{n}/D^{n} \quad [8]$$

then

$$D \times D^{n} = k\ V^{n} \quad [9]$$

and

$$D^{n+1} = k\ V^{n} \quad [10]$$

To determine if the data are in agreement with the Freundlich isotherm, it is more convenient to use a log transformation to make the equation linear, thus:

$$(n+1)\ \ln D = \ln k + n \ln V \quad [11]$$

and

$$\ln D = (\ln k/n+1) + (n \ln V/n+1) \quad [12]$$

A plot of ln D versus ln V yields a straight line, if the data conform to this equation, where the slope (n/n+1), and the intercept (ln k/n+1), are two constants. Once the

two constants have been calculated, desorption can be represented by :

$$D = e^{((\ln k/(n+1)) + (n \ln V)/(n+1))} \quad [13]$$

In the present experiment, data of the WSOC contents of the Malmo and Breton soils, for air-dried and pre-incubated samples, were found to conform to the linear transformation of the desorption equation (Table 4, Fig. 3). Values of n were characteristic of each soil. Values of k, however, were several fold greater for air-dried samples than for pre-incubated samples, therefore reflecting changes in the WSOC pool, under different environmental conditions.

In both soils, isotherms for pre-incubated samples were lower than for air-dried samples , which indicated that the WSOC content was removed from the pool (either by C immobilization and/or by evolution in the form of CO_2-C) as a result of microbial activity when the samples were incubated at field capacity for seven days. For each soil, the amount of WSOC removed from the pool was calculated using the desorption equation and the differences between the intercept values of the desorption isotherms of air-dried and pre-incubated samples. The amounts removed from the WSOC pool were 158 and 45 $\mu g\ g^{-1}$ for the Malmo and Breton soils, respectively. The latter values would reflect that portion of the WSOC pool which became part of, or was utilized by, the soil biomass; 51% and 71% for the Malmo and Breton soils, respectively.

Table 4. Values of n, k and desorption equations calculated from the linear form of the desorption equation , for the Malmo and Breton soils, for air-dried and pre-incubated samples.

Soil	Treatment	n	k	desorption equation
Malmo	Air-dried	0.713	18,106	$e^{(5.72+0.411nV)}$
	Pre-incubated	0.776	7,003	$e^{(4.98+0.431nV)}$
Breton	Air-dried	1.493	31,542	$e^{(4.15+0.591nV)}$
	Pre-incubated	1.421	1,327	$e^{(2.90+0.581nV)}$

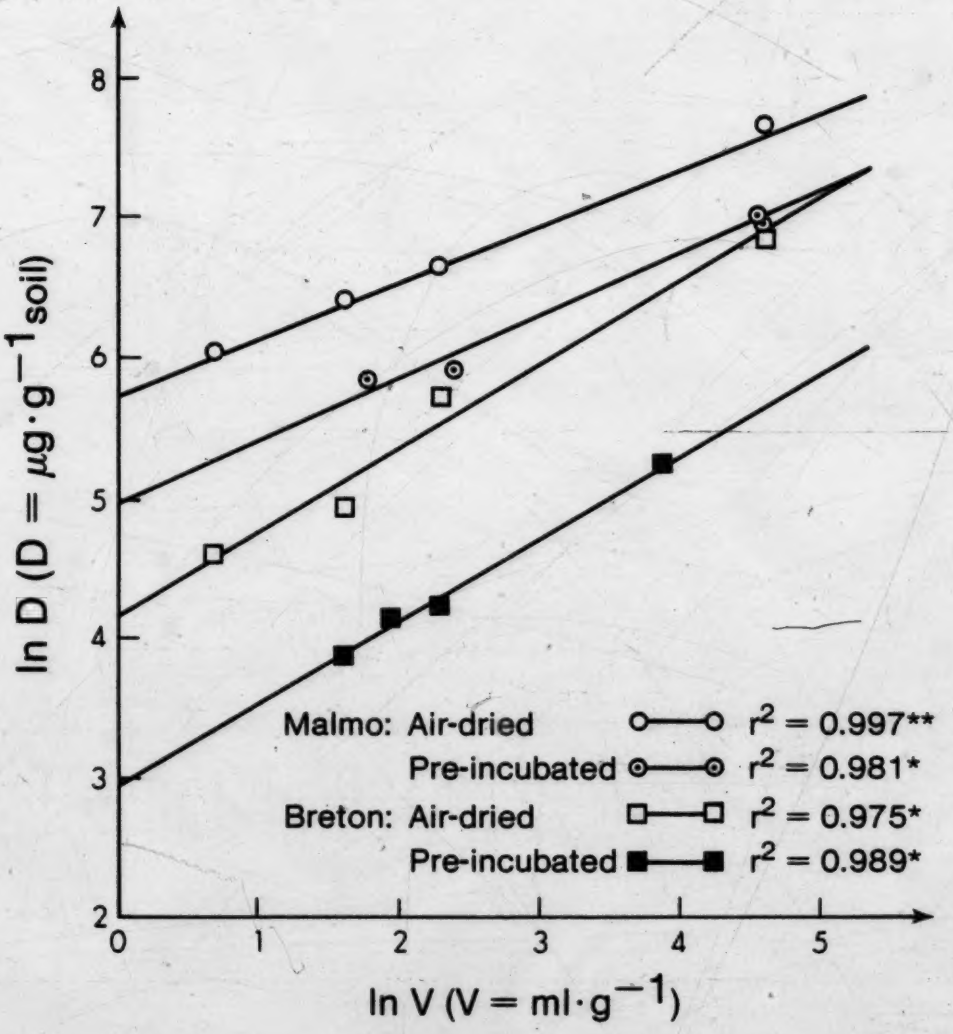

Figure 3. Desorption isotherms of WSOC of air-dried and pre-incubated samples of the Malmo and Breton soils plotted according to the linear form of the desorption equation.

**significant at the 1% level
*significant at the 5% level

4.4.2 Fresh Moist Field Samples

Contents of ninhydrin reactive-N of the extracts of the Malmo and Breton soils varied between 0.7 and 2.8 $\mu g\ g^{-1}$ leucine equivalent. Paul and Schmidt (1960) reported that the total amounts of extractable free amino acid nitrogen in soils rarely exceeds 2 $\mu g\ g^{-1}$. Bremner(1967) indicated that free amino acids are rapidly decomposed when added to soils. Also, he indicated that although the quantities of such compounds may be very small in soil, such compounds may be of special significance in the rhizosphere, where roots, soil and microorganisms are intimately interrelated.

It can be concluded that ninhydrin-reactive materials found in water extracts of field moist samples were relatively small and varied little between the two soils examined.

4.5 Summary

1. The present study indicates that desorption of WSOC can be described by a modified Freundlich isotherm, which can be used to characterize a soil.

2. Parameters of the desorption equation obtained for air-dried and pre-incubated samples can be used to determine the portion of the WSOC pool utilized by microbial activity as well as the effect of drying and other perturbation of soil. 158 and 45 $\mu g\ g^{-1}$ of WSOC were removed from air-dried samples of the Malmo and Breton soils, respectively, during a 7 day incubation period at field capacity.

3. The differences of the WSOC contents of air-dried and pre-incubated samples of the Malmo and Breton soils were highly significant.

4. Ninhydrin-reactive materials found in water extracts of field moist samples were relatively small and varied little between the two soils examined.

5. Magnitude of Assimilatory Nitrate Reduction

5.1 Introduction

In studies on denitrification, the question always arises whether NO_3^--N disappearence can be attributed to assimilatory nitrate reduction (N immobilization) or denitrification (dissimilatory nitrate reduction). Because assimilatory nitrate reduction retains nitrogen in the soil rather than losing it as gaseous reduction product, it is important to determine to what extent this process occurs in the soil, in order to assess its relative impact on the nitrogen cycle.

Consequently, in the following experiment labelled ^{15}N fertilizer was used to determine the extent of NO_3^--N immobilization in the Malmo soil, compared to losses by denitrification.

5.2 Experimental Design

Soil samples were treated with 192 $\mu g\ g^{-1}$ of $Ca(^{15}NO_3)_2$ with 1.35% excess ^{15}N. The samples were incubated for 15 days as outlined in section 3.3. Each treatment was replicated four times. Determinations of NO_3^--N, NO_2^--N, NH_4^+-N and total N were performed at time 0 and after 5 and 15 days of incubation.

In this experiment, for each nitrogen determination, 30 ml of distillate were collected, titrated and an additional 1 ml of titration acid added to further acidify the samples.

The samples were evaporated to dryness at 60 C and stored for isotope ratio analysis in the mass spectrometer. The ammonia in the extracts was oxidized by an alkaline solution of lithium hypobromite to nitrogen gas in vacuum, and admitted to the mass spectrometer to determine $^{14}N/^{15}N$ ratios (Porter and O'Dean, 1977). The reaction proceeds as shown in the following chemical equation:

$$2NH_3 + 3OBr^- \longrightarrow N_2 + 3H_2O + 3Br^- \qquad [1]$$

Isotope ratio analysis were performed on a micromass 602 Isotope Ratio Mass Spectrometer.

The amounts of labelled ^{15}N fertilizer remaining in the soil in the form of NH_4^+-N, NO_3^--N, NO_2^--N and Organic N were calculated using the expressions as shown in the Appendix (Tables C and D).

5.3 Results and Discussion

In this experiment the NO_3^--N content decreased from 195 to 5 μg g^{-1} after 5 days of incubation and to less than 1 μg g^{-1} after 15 days. The NO_2^--N content decreased from 6 to 1 μg g^{-1} after 5 days of incubation and completely disappeared after 15 days of incubation (Table 5).

The labelled ^{15}N present in the form of ^{15} NO_3^--N was 165 μg g^{-1} at the beginning of the incubation and it decreased to 3.4 μg g^{-1} after 5 days and to less than 1 μg g^{-1} after 15 days of incubation. No ^{15}N was detected in the form of NO_2^--N (Table 6).

Table 5. Mineral N contents in the Malmo soil incubated under water saturated conditions, at different intervals of time.

Time	NH_4^+-N	NO_3^--N	NO_2^--N
days	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
0	8.9 ± 0.0	195.3 ± 9.0	6.4 ± 1.8
5	92.0 ± 5.6	5.6 ± 3.0	1.2 ± 1.6
15	54.0 ± 3.5	0.3 ± 0.7	0.0

± indicates standard deviation.

Table 6. Labelled ^{15}N contents present in the Malmo soil as NH_4^+-N, NO_3^--N, NO_2^--N, and Organic N, at different intervals of time, when incubated under water saturated conditions.

Time	NH_4^+-N	NO_3^--N	NO_2^--N	Organic-N
days	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
0	0.6 ± 0.4	165.1 ± 24.8	0	26.4 ± 29.7
5	7.6 ± 7.7	3.4 ± 3.0	0	9.7 ± 2.1
15	3.9 ± 3.1	0.2 ± 1.0	0	10.2 ± 1.4

± indicates standard deviation.

Table 7. Percent of labelled ^{15}N present as NH_4^+-N, NO_3^--N and Organic-N in the Malmo soil, at different intervals of time, when incubated under water saturated conditions.

Time	NH_4^+-N	NO_3^--N	Organic-N
days	%	%	%
0	0.3	86.0	13.7
5	3.9	1.7	5.0
15	2.0	0.1	5.2

The NH_4^+-N content increased from 9 to 92 μg g^{-1} after 5 days of incubation, then it decreased to 54 μg g^{-1} after 15 days. However, the total amount of labelled ^{15}N present in the form of NH_4^+-N corresponded only to 7.6 μg g^{-1} after 5 days of incubation and to 3.9 μg g^{-1} after 15 days. This indicated that after 5 days of anaerobic incubation, only 9% of the total NH_4^+-N present was derived from the initially added labelled ^{15} NO_3^--N. Furthermore, after 15 days of incubation only 7% of the total NH_4^+-N present was derived from the labelled fertilizer. In light of these results, the increased mineralization can possibly be explained in terms of shifts in the microbial population, causing elimination and decomposition of microbial cells of decaying soil biomass (Tables 5 and 6).

The ^{15}N present in the form of organic N was 26.4 ± 29.7 μg g^{-1} at the beginning of the incubation and decreased to 9.7 ± 2.1 and 10.2 ± 1.4 μg g^{-1} , after 5 and 15 days of incubation, respectively (Table 6).

With these results, the percentages of the initially added labelled ^{15}N present in the form of NH_4^+-N, NO_3^--N and organic N were calculated. After 5 days of incubation, 5% of labelled ^{15}N was present in the form of organic N, 4% was in the ammonium form and less than 2% remained in the form of NO_3^--N. After 15 days of incubation, 5.2% of the originally added ^{15}N was present as organic N, 2% was in the NH_4^+-N form and less than 1% in the form of NO_3^--N (Table 7).

Consequently, the amounts of labelled $^{15}NO_3$-N lost from the soil were equivalent to 89.2% and 92.6% after 5 and 15 days of incubation, respectively. These losses were attributed to denitrification.

These findings are in agreement with other investigations. Woldendorp(1963) in an experiment designed to clarify the conditions under which nitrate was reduced by *Bacillus licheniformis* and *B. subtilis* to ammonium, found that the accumulation of this compound occurs only in the cells grown aerobically in the absence of ammonium. Furthermore, the same author, used tagged NO_3-N to investigate the capacity of permanent grassland sod derived from sandy soil, to reduce nitrate to ammonium, when incubated under anaerobic conditions. The results of this experiment indicated that the formation of ammonium from nitrate was negligible and hardly exceeded the 1% level at all stages. In spite of that the total amount of ammonium in the soil was clearly higher upon addition of nitrate.

5.4 Summary

The ^{15}N data of this investigation indicate that NO_3-N disappearance by bacterial reduction to ammonium or immobilization was minimal. Increments in the non-labelled NH_4 content can be explained mainly in terms of deamination activity occurring to disrupted cells of the microbial soil biomass. NO_3-N disappearence can be attributed mainly to denitrification.

6. Nitrate Losses in Relation to the Water Soluble Organic Carbon Supply

The role of organic matter as a reductant in denitrification is well recognized (Bremner and Shaw,1958; Woldendorp, 1963; Delwiche and Bryan,1976). Moreover, it has been found that the supply of readily decomposable organic matter or water-soluble carbon provides a good index of the soil potential for denitrification(Burford and Bremner,1975). Changes in soluble C content during denitrification, however, have not been reported.

In order to establish the relationship between NO_3^--N losses and the WSOC contents in the Breton and Malmo soils, a set of experiments were performed in which NO_3^--N losses and concurrent changes in WSOC content were followed under saturated moisture conditions. These results are reported in sections 6.1, 6.2, 6.3, and 6.4. Also, the effect of high NO_3^--N concentrations and mixtures of NO_3^--N and NO_2^--N on denitrification was examined in these soils; the results are reported in section 6.5.

6.1 Nitrate Losses in the Malmo and Breton Soils in Relation to WSOC Content

6.1.1 Experimental Design

Air dried soil samples were incubated as outlined in section 3.3.

1. Soils:

a. Malmo SiCl.

b. Breton L.

2. Treatments:

a. Control (No NO_3^--N addition).

b. Addition of NO_3^--N at a rate of 200 μg g^{-1} in the Malmo soil and at a rate of 130 μg g^{-1} in the Breton soil.

3. Incubation period: 10 days under anaerobic conditions

4. WSOC determinations: persulfate oxidation (Hu *et al.*, 1972), using a water:soil ratio of extraction of 10:1.

5. Determinations of NO_3^--N, NO_2^--N, NH_4^+-N, the denitrifying population , CO_2-C evolution rates and WSOC contents were made at different intervals of time.

6.1.2 Results and Discussion

Malmo Soil

In the control treatment the NH_4^+-N,the NO_3^--N and the NO_2^--N contents remained unchanged after 10 days of incubation (Table 8), whereas the WSOC content of the control decreased from 556 to 366 μg g^{-1}. The denitrifying population fluctuated between $1.5x10^8$ and $1.7x10^9$ organisms per gram of soil during the first 4 days of incubation and increased later to $1.7x10^8$. The CO_2-C evolution rate 28 μg g^{-1} day^{-1}, when measured after 2, 7 and 10 days of incubation.

When 200 μg g^{-1} NO_3^--N were applied to the soil, the NH_4^+-N and the NO_2^--N contents remained unchanged The NO_3^--N

content decreased from 201 to 2 $\mu g\ g^{-1}$. The WSOC content decreased abruptly from 556 to 141 $\mu g\ g^{-1}$ within the first day of incubation and then increased to 267 $\mu g\ g^{-1}$ after 10 days. The population of denitrifiers fluctuated between $1.5x10^3$ and $1.6x10^4$ organisms per gram of soil during the incubation period. The CO_2-C evolution rate was 12 $\mu g\ g^{-1}$ per day after 2 days of incubation and increased to 18 $\mu g\ g^{-1}$ per day after 7 and 10 days of incubation.

In general, replacement of some of the depleted WSOC was detected during the course of the incubation in both treatments.

Estimates of the denitrifying population size indicate that the number of denitrifiers declined and later increased by a factor of 10.

The rate of CO_2-C evolution was greater in the control treatment than when 200 $\mu g\ g^{-1}$ of NO_3^--N was applied to the soil and the denitrifier population size was greater by a factor of 10 in the latter. At the same time, the amount of WSOC remaining in the soil was smaller in the NO_3^--N amended soil than in the control, after 10 days of incubation. This must be due to the effect of growth of different microbial populations using iron or manganese as electron electron acceptors under anaerobic conditions, in the absence of NO_3^-, and also using other organic compounds besides the WSOC, which remained rather stable during the incubation. In the absence of NO_3^-, manganese dioxide can function as an electron acceptor in anaerobic respiration at an oxidation

Table 8. NH_4^+-N, NO_3^--N, NO_2^--N, WSOC, CO_2-C evolution rate and denitrifier population of initially air dried Malmo soil samples moistened to saturation and incubated at 28 C .

Treatment	Time	NH_4^+-N	NO_3^--N	NO_2^--N	WSOC	CO_2-C evol.	Denitrifiers
	days	ug g^{-1}	ug g^{-1}	ug g^{-1}	ug g^{-1}	ug g^{-1} d^{-1}	organisms g^{-1}
Control	0	53 ± 6	1 ± 2	0	556 ± 35		1.5 ± 1 x 10^4
	1	50 ± 9	0	0	336 ± 40		
	2	49 ± 2	16 ± 3	0	317 ± 45	28	5.7 ± 2 x 10^3
	4	42 ± 6	0	4 ± 4	333 ± 50		1.7 ± 3 x 10^3
	7	61 ± 13	1 ± 1	3 ± 2	439 ± 52	28	
	10	65 ± 4	2 ± 1	2 ± 2	366 ± 40	28	1.7 ± 2 x 10^4
200 ug g^{-1} of NO_3^--N	0	53 ± 6	201 ± 12	0	556 ± 35		1.5 ± 1 x 10^4
	1	51 ± 5	185 ± 16	0	141 ± 26		
	2	52 ± 9	132 ± 16	0	197 ± 30	12	1.6 ± 3 x 10^4
	4	36 ± 3	56 ± 11	0	211 ± 35		2.0 ± 1 x 10^4
	7	55 ± 2	8 ± 6	0	217 ± 32	18	
	10	59 ± 8	2 ± 2	0	267 ± 42	18	1.2 ± 4 x 10^3

± indicates standard deviation

Table 9. NH_4^+-N, NO_3^--N, WSOC, CO_2-C evolution rate and denitrifier population of initially air dried Breton soil samples moistened to saturation and incubated at 28 C .

Treatment	time	NH_4^+-N	NO_3^--N	WSOC	CO_2-C evol.	Denitrifiers
	days	ug g^{-1}	ug g^{-1}	ug g^{-1}	ug g^{-1} d^{-1}	organisms g^{-1}
Control	0	3 ± 2	0	58 ± 20		1.9 ± 1 x 10^3
	1	4 ± 1	0	52 ± 32		
	2	9 ± 3	0	41 ± 15	4.6	
	5	6 ± 2	1 ± 1	87 ± 12	4.5	4.9 ± 2 x 10^3
	10	9 ± 4	2 ± 1	69 ± 30	13.9	2.4 ± 1 x 10^3
132 ug g^{-1} of NO_3^--N	0	2 ± 2	132 ± 12	58 ± 15		2.4 ± 1 x 10^3
	1	4 ± 1	118 ± 9	42 ± 30		
	2	7 ± 1	107 ± 5	32 ± 18	3.9	
	5	5 ± 1	103 ± 4	45 ± 21	3.2	2.8 ± 3 x 10^4
	10	10 ± 1	103 ± 2	34 ± 12	9.3	4.6 ± 2 x 10^4

± indicates standard deviation .

level comparable to that of nitrate. The presence of nitrate in flooded soils retards manganese reduction, suggesting that the facultative anaerobic bacteria uses nitrate preferentially as an electron acceptor. About the time of maximum accumulation of reduced manganese compounds in flooded soils, the amount of ferrous iron starts to increase(Yoshida, 1975). In experiments by Asami and Takai, quoted by Yoshida(1975), the amount of iron reduced was highly correlated with the amount of carbon dioxide produced for about two weeks after flooding. Also, the addition of nitrate has been found to decrease the activity of the iron-reducing bacteria (Yoshida, 1975).

Considering that in the NO_3^--N amended soil, 289 $\mu g\ g^{-1}$ of WSOC was depleted (WSOCdep) during the 10 days incubation period and that the total amount of CO_2-C evolved (CO_2-Cevol)for the same period was 162 $\mu g\ g^{-1}$, the carbon conversion efficiency (%Cce) into microbial biomass may be estimated, using the following expression:

$$\%Cce = ((WSOCdep - CO_2\text{-}Cevol)/WSOCdep\ )\ 100 \qquad [1]$$

This results in an estimated C conversion efficiency of 44%, when NO_3^--N was added to the soil. Values between 40 and 60 percent were reported for aerobic soils by McGill *et al.* (1974). In the control treatment, however, the net WSOC depletion was smaller than the total amount of CO_2-C evolved in 10 days of incubation, 190 and 280 $\mu g\ g^{-1}$, respectively. The latter would suggest that besides the partial

utilization of the WSOC pool, other sources of carbon were utilized in the unamended soil or the WSOC pool was raplidly replenished. Also, the higher CO_2-C evolution rate of the unamended soil, together with a denitrifier population only 1/10 that in the amended soil would suggest a lower efficiency of utilization of carbon sources may have occurred in the unamended soil compared to the amended one.

In this experiment, the denitrification rate did not follow second order kinetics (Figure 4), because the line, although straight, did not pass through the origin, and the C content increased with consistency, rather than decreasing with time. The rate constant, k, was determined from the slope of the regression of ln((Bo/Ao)(At/Bt)) on t(days), according to equation [22] (page 24) in which k = m/(Ao - 2Bo) and was found to be 0.003651 g ug^{-1} day^{-1}. The second order regression was:

$$Y=-1.6745 + 0.5622X \text{ and } r^2= 0.992 \qquad [2]$$

The large negative intercept suggests some process in addition to simple second order kinetics as described in section 2.7.

Using the same data, the first order rate constant, k, with respect to NO_3^--N was determined from the slope of the regression of ln NO_3^--N remaining (in $\mu g\ g^{-1}$) on t (days), based on the first order equation:

$$\ln NO_3\text{-}Nt = \ln NO_3\text{-}No - kt \qquad [3]$$

The resulting k calculated was equal to 0.489 d^{-1}, with the first order regression equation as follows:

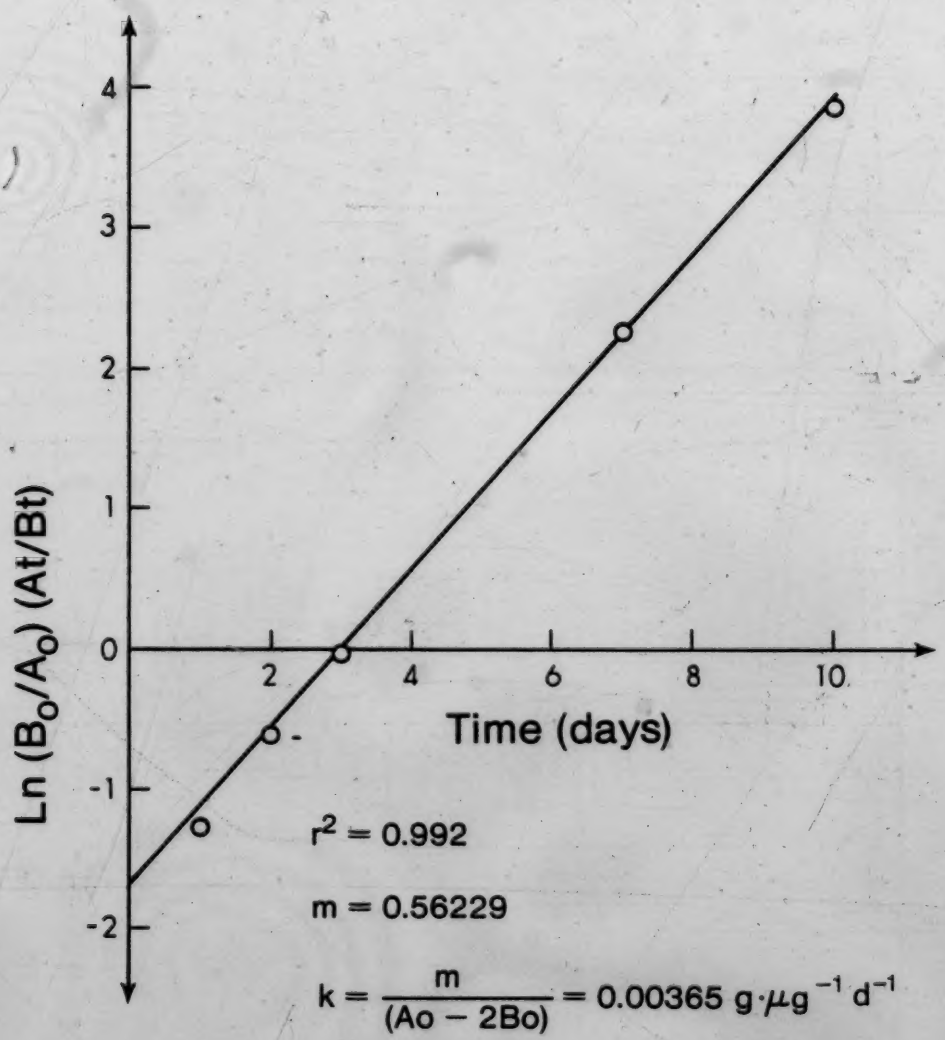

Figure 4. Representation of the change in C and NO_3^-–N with time, where A represents C and B represents NO_3^-–N in the Malmo soil.

$Y = 5.659 - 0.489X$ and $r^2 = 0.981$ [4]

Considering that the value of r^2 calculated for both equations were similar, any of them could be used to represent NO_3^--N losses in the Malmo soil. The first order plot gives a better representation of the system because of its large r^2 value and positive intercept.

Breton Soil

In the control treatment the NH_4^+-N, the NO_3^--N and the WSOC contents varied slightly during the 10 days of incubation. The CO_2-C evolution rate increased from 4.6 to 13.9 $\mu g\ g^{-1}\ d^{-1}$, after 2 and 10 days of incubation. During this period, the denitrifying population fluctuated between 1.9×10^3 and 4.9×10^3 organisms g^{-1} (Table 9).

When 132 $\mu g\ g^{-1}$ of NO_3^--N were applied to the soil, the NH_4^+-N content increased slightly from 3 to 10 $\mu g\ g^{-1}$ and the NO_3^--N content decreased from 132 to 103 $\mu g\ g^{-1}$ and remained constant. Therefore, only 29 $\mu g\ g^{-1}$ of the applied NO_3^--N were lost in the 10 days of incubation. The WSOC content decreased from 58 to 34 $\mu g\ g^{-1}$, after 10 days (Figure 5). The WSOC content was approximately one tenth of that found in the Malmo soil. The denitrifying population of the amended soil increased from 1.9×10^3 to 4.6×10^4 organisms g^{-1}, during the incubation period. The CO_2-C evolution rate increased from 3.9 to 9.3 $\mu g\ g^{-1}\ d^{-1}$, after 2 and 10 days of incubation.

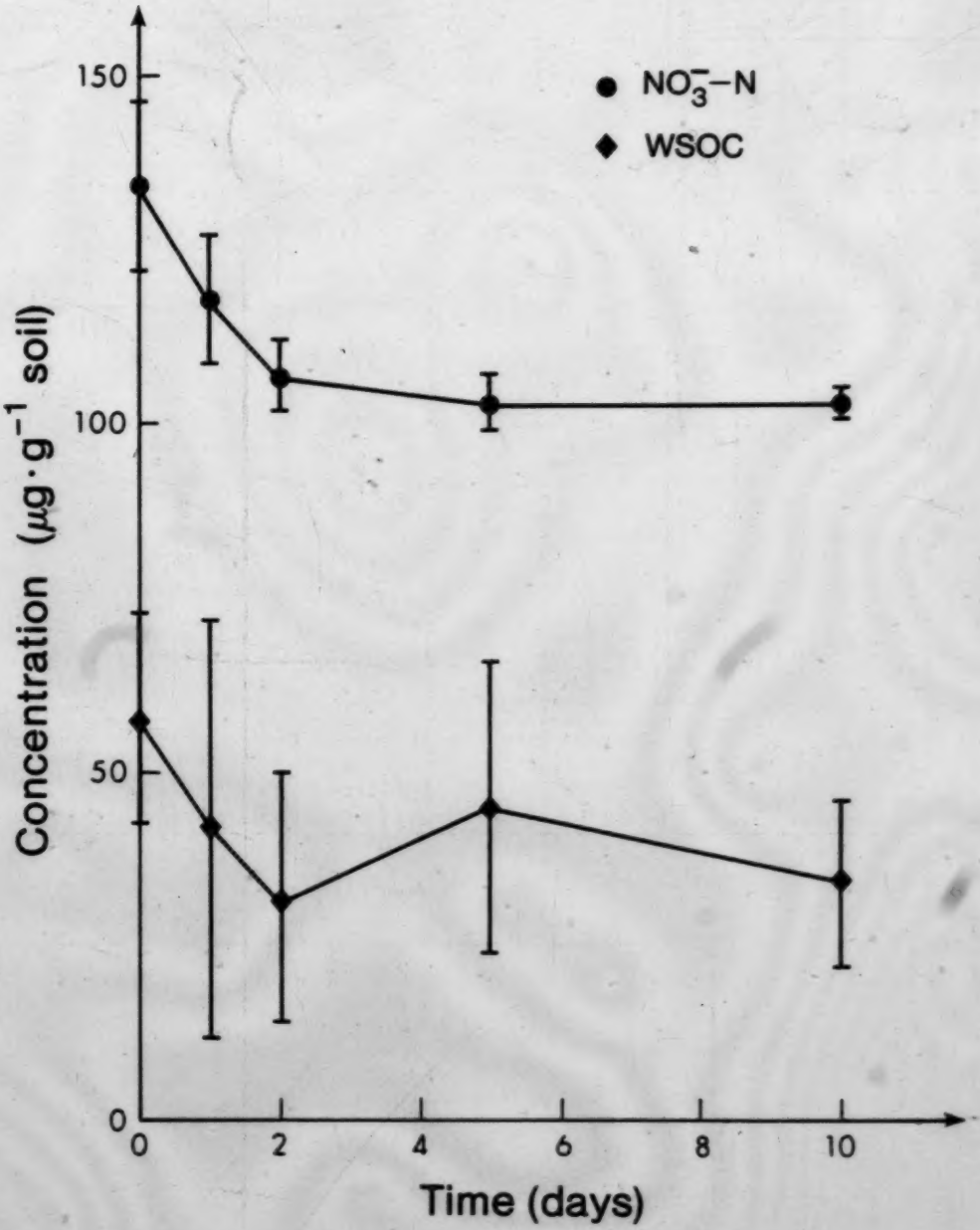

Figure 5. NO_3^-–N and WSOC contents and standard deviation of anaerobically incubated Breton soil samples, at different intervals of time.

In general, in both treatments, small increments in the NH_4^+-N contents as well as replacement of some of the depleted WSOC were observed. As in the Malmo soil, the rate of evolution of CO_2-C was greater in the control treatment than when NO_3^--N was present.

NO_3^--N losses in these experiments did not follow first or second order kinetics. This could be attributed to the small losses of NO_3^--N (up to 28 $\mu g\ g^{-1}$) in the amended soil and to the relatively small WSOC content of the soil. After two days of incubation the NO_3^--N losses completely ceased in the Breton soil. On this basis, the lower denitrification capacity of the Breton soil is mainly attributed to a lower amount of the WSOC in this soil.

6.1.3 Summary

Results indicated that 200 $\mu g\ g^{-1}$ of NO_3^--N were denitrified from air dried samples of the Malmo soil after 10 days of incubation under saturated moisture conditions and during the same period 290 $\mu g\ g^{-1}$ of WSOC were depleted. Under similar incubation conditions, however, air dried samples of the Breton soil denitrified only 29 $\mu g\ g^{-1}$ of NO_3^--N and denitrification ceased after 2 days of incubation, when the WSOC content dropped from 58 to 32 $\mu g\ g^{-1}$. NO_3^--N losses in the Breton samples were equivalent to 21% of the originally applied NO_3^--N.

Smaller losses of NO_3^--N in the Breton samples were attributed to the low indigenouos WSOC content, which was

1/10 of that found in the Malmo soil samples.

6.2 Nitrate Losses in Pre-incubated Samples of the Malmo and Breton Soils and its Relationship to the WSOC Content

In section 4 of this paper, it was found that pre-incubated samples of the Malmo and Breton soils have a lower content of WSOC than air-dried samples. The objective of this experiment was to determine NO_3-N losses in pre-incubated samples, which contain lower amounts of WSOC.

6.2.1 Experimental Design

Soil samples were pre-incubated as outlined in section 3.3. Two studies were designed for each soil.

Malmo Soil:

a. In study No 1, the NO_3-N was applied at a rate of 124 $\mu g\ g^{-1}$ and the soil samples were incubated anaerobically for 14 days.

b. In study No 2, the NO_3-N was applied at a rate of 196 $\mu g\ g^{-1}$ and the soil samples were incubated anaerobically for 24 days.

Breton Soil:

a. In study No 3, the NO_3-N was applied at 392 $\mu g\ g^{-1}$ and the soil samples were incubated anaerobically

for 10 days.

b. In study No 4,the NO_3^--N was applied at rate of 329 μg g^{-1} and the soil samples were incubated anaerobically for 10 days.

Each study was replicated 3 times and determinations of NO_3^--N, NO_2^--N, and NH_4^+-N were made using the methods outlined in section 3.3. The WSOC content determinations used the Mebius Method(1960) and the water:soil extraction ratio was 10:1.

6.2.2 Results and Discussion

Malmo soil

In study No 1, the NH_4^+-N content increased from 70 to 109 μg g^{-1} after 14 days (Table 10). The NO_3^--N content decreased from 124 to 13 μg g^{-1} after the second day of incubation and it completely disappeared after the fifth day. The NO_2^--N content increased from 0 up to 14 μg g^{-1} after the second day and then completely disappeared. The WSOC content decreased sharply from 601 to 192 μg g^{-1} after the second day of incubation, increasing up to 340 μg g^{-1} after 14 days (Table 10).

In study No 2, the NH_4^+-N content increased from 87 to 145 μg g^{-1}. The NO_3^--N content decreased from 196 to 2 μg g^{-1} after the second day of incubation and only slight changes were detected thereafter (± 1 μg g^{-1}). The NO_2^--N content decreased from 12 to 1 μg g^{-1} and varied slightly during the rest of the incubation period. The WSOC content decreased

from 492 to 244 μg g^{-1} after 17 days of incubation and it increased up to 598 μg g^{-1} after 24 days of incubation.

Therefore, in the pre-incubated samples of the Malmo soil, most of the NO_3^--N disappeared after the second day of incubation with a concomitant decrease in the WSOC content. Thereafter, the WSOC content steadily increased, so that in both studies, the WSOC content increased up to 340 μg g^{-1} after 14 days. In study No 2, where the samples were incubated for 24 days, the WSOC content was even higher after 24 days than the original amount present.

Breton soil

In study No 3, the NH_4^+-N content increased from 0 to 29 μg g^{-1} after 24 days. During this period , the NO_3^--N content decreased from 392 to 310 μg g^{-1} (Table 11). The NO_2^--N content increased from 16 to 86 μg g^{-1} during the first two days of incubation and thereafter it steadily decreased to 32 μg g^{-1} after 24 days. The WSOC content decreased from 110 to 19 μg g^{-1} during the first two days of incubation and varied between 10 and 30 μg g^{-1} during the rest of the incubation period. In study No 4, the NH_4^+-N content remained unchanged after 10 days of incubation. The NO_3^--N decreased from 329 to 313 μg g^{-1} after 10 days. The NO_2^--N content increased from 36 up to 79 μg g^{-1} after two days and decreased to 12 μg g^{-1} after 10 days. The WSOC content was extremely low during the whole incubation period, varying between 20 and 27 μg g^{-1} (Table 11).

Table 10. NH_4^+-N, NO_3^--N, NO_2^--N and WSOC content of pre-incubated samples of the Malmo Soil, when incubated under water saturated conditions, at 28 C.

	Time days	NH_4^+-N $\mu g\ g^{-1}$	NO_3^--N $\mu g\ g^{-1}$	NO_2^--N $\mu g\ g^{-1}$	WSOC $\mu g\ g^{-1}$
Study No. 1	0	70 ± 7	124 ± 3	0	601 ± 30
	2	72 ± 7	27 ± 2	14 ± 4	192 ± 35
	5	96 ± 2	0	0	254 ± 23
	7	95 ± 4	0	0	190 ± 7
	9	97 ± 5	<1	0	209 ± 32
	14	109 ± 4	<1	0	340 ± 26
Study No. 2	0	87 ± 2	196 ± 8	12 ± 2	492 ± 32
	2	100 ± 4	2 ± 1	1 ± 1	320 ± 20
	5	106 ± 12	<1	1 ± 1	288 ± 25
	10	130 ± 8	3 ± 2	0	364 ± 20
	14	125 ± 16	2 ± 1	0	340 ± 18
	17	129 ± 10	3 ± 2	2 ± 1	244 ± 23
	24	145 ± 16	2 ± 1	0	598 ± 35

± indicates standard deviation.

Table 11. NH_4^+-N, NO_3^--N, NO_2^--N and WSOC content of pre-incubated samples of the Breton soil, when incubated under water saturated conditions , at 28 C.

	Time days	NH_4^+-N $\mu g\ g^{-1}$	NO_3^--N $\mu g\ g^{-1}$	NO_2^--N $\mu g\ g^{-1}$	WSOC $\mu g\ g^{-1}$
Study No. 3	0	0	392 ± 11	16 ± 4	110 ±15
	2	25 ± 12	317 ± 12	86 ± 16	19 ± 9
	5	26 ± 2	281 ± 7	79 ± 7	10 ± 2
	10	28 ± 4	322 ± 8	21 ± 5	25 ± 12
	14	27 ± 2	291 ± 9	45 ± 12	30 ± 3
	17	27 ± 8	304 ± 7	46 ± 13	18 ± 12
	24	29 ± 6	310 ± 12	32 ± 7	16 ± 3
Study No. 4	0	23 ± 3	329 ± 8	36 ± 9	20 ± 2
	1	23 ± 2	312 ± 7	47 ± 12	13 ± 4
	2	25 ± 5	285 ± 12	79 ± 11	25 ± 15
	3	25 ± 1	329 ± 9	22 ± 9	24 ± 8
	10	26 ± 2	313 ± 11	12 ± 7	27 ± 4

± indicates standard deviation.

In general, in studies No 3 and No 4, slight changes in the NH_4^+-N and WSOC content occurred during the incubation period. Also, the applied NO_3^--N decreased by 82 and 16 μg g^{-1} in studies No 3 and No 4, respectively. Both studies indicated that the NO_3^--N was mainly reduced to NO_2^--N, remaining principally as such,without being further reduced. This was probably due to the lack of hydrogen donors or readily decomposable carbon sources, needed to further reduce the NO_2^--N to gaseous N forms.

6.2.3 Summary

This set of studies showed that pre-incubated samples of the Malmo soil possess a potential capability to readily denitrify up to 200 μg g^{-1} of NO_3^--N in a two day period. These NO_3^--N losses were associated with changes in the WSOC content during the same period. These findings were consistent with results reported in section 6.1 of this paper, in which a similar amount of NO_3^--N disappeared from air dried samples of the Malmo soil. These results were also in agreement with results reported by Gould and McCready (1981), who found that extensive denitrification occurred in a Black Chernozemic soil when the soil was incubated with a water content equivalent to twice field capacity. Under similar experimental conditions, they found that in a Gray Luvisol, no NO_3^--N losses occurred. On the contrary in the present study, up to 82 μg g^{-1} of NO_3^--N were lost from the

Breton soil samples, which were equivalent to 20% of the originally applied NO_3^--N, and the WSOC dropped from 110 to 10 $\mu g\ g^{-1}$. In section 6.1, NO_3^--N losses were equivalent to 20% of the originally applied NO_3^--N.

Therefore, it was concluded that the pre-incubation treatment of the Malmo and Breton soils, did not alter their capacities to denitrify, which were closely related to their different supplies of soluble carbon, as previously determined in section 6.1 of this paper.

6.3 Effect of the Addition of a Carbon Source on Nitrate Losses in the Breton Soil

In the previous experiments, it was found that the WSOC present in the Breton soil was scarce and only partial losses of NO_3^--N would occur. Therefore, the present experiment was conducted in order to determine if the lack of organic carbon was the factor preventing denitrification in the Breton soil.

6.3.1 Experimental Design

Soil samples were pre-incubated and incubated following the procedures as outlined in section 3.3. This experiment consisted of two studies, replicated three times each, in which the effect of the application of 600 $\mu g\ g^{-1}$ of glucose-C was examined using pre-incubated Breton soil samples, and two rates of application NO_3^--N, each followed by incubation under saturated moisture conditions.

In study No 1, the NO_3^--N was applied at a rate of 125 μg g^{-1} and the samples were incubated anaerobically for 14 days.

In study No 2, the NO_3^--N was applied a rate 196 μg g^{-1} and the samples were incubated anaerobically for 24 days.

The WSOC determinations were made on the Beckman 915-B analyzer and extracts were obtained using a water:soil ratio of 10:1.

6.3.2 Results and Discussion

In study No 1, the NO_3^--N decreased from 125 to 1 μg g^{-1} after two days of incubation (Table 12). Later, small increments in the NO_3^--N content were detected which varied between 1 and 5 μg g^{-1}. The NO_2^--N content decreased from 3 to less than 1 μg g^{-1} after two days and it was not detected thereafter. The WSOC content decreased from 620 to 361 μg g^{-1} after two days and it increased to 490 μg g^{-1} after 14 days. The NH_4^+-N content increased from 19 to 28 μg g^{-1} after 14 days of incubation.

In study No 2, the NO_3^--N content decreased from 196 to 1 μg g^{-1} after two days of incubation and it was not detected thereafter. The NO_2^--N decreased from 16 to less than 1 μg g^{-1} after two days and completely disappeared thereafter. The WSOC decreased from 620 to 293 μg g^{-1} after two days. From then on, the WSOC content increased 427 μg g^{-1} after 14 days and decreased again to 68 μg g^{-1} after 24 days. The NH_4^+-N content increased from 1 to 49 μg g^{-1} after

Table 12. NH_4^+-N, NO_3^--N, NO_2^--N and WSOC content and standard deviation in pre-incubated samples of the Breton soil amended with NO_3^--N and 600 μg g^{-1} glucose-C and incubated under water saturated conditions.

	Time	NH_4^+-N	NO_3^--N	NO_2^--N	WSOC
	days	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}
Study No 1	0	19 ± 6	125 ± 5	3.3 ± 1	620 ± 40
	2	6 ± 5	<1	<1	361 ± 25
	5	10 ± 4	<1	0	389 ± 32
	7	23 ± 11	4 ± 3	0	309 ± 36
	9	22 ± 8	5 ± 4	0	420 ± 18
	14	28 ± 4	2 ± 1	0	490 ± 25
Study No 2	0	1 ± 1	196 ± 11	16 ± 3	630 ± 40
	2	38 ± 4	<1	<1	293 ± 11
	5	37 ± 1	<1	<1	300 ± 23
	10	37 ± 7	0	0	364 ± 40
	14	49 ± 1	0	0	427 ± 6
	17	36 ± 9	0	0	92 ± 33
	24	31 ± 7	0	0	68 ± 35

± indicates standard deviation.
Study No 1: amendment of 125 μg NO_3^--N g^{-1}
Study No 2: amendment of 196 μg NO_3^--N g^{-1}

14 days of incubation, and from then on it steadily decreased to 31 $\mu g\ g^{-1}$ after 24 days (Table 12).

In general, for both studies, slight fluctuations in the NH_4^+-N content were detected during the whole incubation period. In both studies, all the NO_3^--N originally applied disappeared within two days of incubation with a simultaneous decrease in the WSOC content, usually followed by an increase in WSOC content. These findings agreed with results reported by Burford and Bremner(1975), who studied the effect of glucose on denitrification; when samples were incubated anaerobically at 20 C for 7 days after treatment with nitrate (0.4 mg NO_3^--N/g), these soils denitrified only 4 to 17 % of the added nitrate. When incubated under the same conditions plus the addition of 2 mg glucose-C g^{-1} soil, the soils denitrified all the added nitrate (no nitrate-N was detected after 7 days, and 96-99 % of NO_3^--N lost was recovered as N_2 and N_2O). More recently, Ryden and Lund(1980) suggested that denitrification in the field may more often be controlled by available C rather than NO_3^--N concentration. Also, Limmer and Steele(1982), found that denitrification potential increased with addition of glucose amendments to a soil from New Zealand.

Rolston *et al.* (1978), in field measurements of denitrification using labelled ^{15}N, found that the addition of manure to the soil greatly increased the rate and amount of denitrification. They also found that the presence of a crop root system (compared to an uncropped field) had a

large influence on denitrification, because it provided a carbon supply.

Nevertheless, Gould and McCready (1982), did not observe an increase in denitrification in a Grey Luvisol, when the soil was treated with 300 $\mu g\ g^{-1}$ of glucose-C, suggesting that either the indigenous microflora were incapable of denitrification or some other chemical factor may have been limiting denitrification.

6.3.3 Summary

In sections 6.1 and 6.2 of this paper it was determined that the Breton soil samples denitrified up to 20% of the initially added NO_3-N. In the present experiment, with the addition of 600 μg glucose-C g^{-1}, the Breton soil samples readily denitrified 125 and 196 μg NO_3-N g^{-1}, for studies No 1 and 2 respectively. These losses corresponded to 100% of the added NO_3-N in each study. Therefore, it was concluded that the critical factor limiting the denitrification in the Breton soil was the low native WSOC content. When glucose was edded, the NO_3-N were almost complete.

6.4 Continuous Nitrate losses in Relation to the Remaining WSOC Supply in the Malmo soil

In previous experiments, it was determined that up to 200 $\mu g\ g^{-1}$ of NO_3-N could be lost from the Malmo soil, and these losses were found to be related to the WSOC content. However, it was not determined to what extent these losses

could be sustained, considering their relationship to the indigenous WSOC pool. Thus, the objective of this experiment was to study how long the WSOC present in the Malmo soil would be able to support continuous losses of NO_3^--N.

6.4.1 Experimental Design

This experiment was conducted with samples taken of the Ap horizon of a Malmo SiCl soil which was collected in the spring of 1980 from a field with barley stubble, at the the University of Alberta Ellerslie farm.

Air-dried soil samples were treated with 200 $\mu g\ g^{-1}$ of NO_3^--N and incubated under water saturated conditions, following the procedures as outlined in section 3.3. Samples were replicated three times each. Determinations of NO_3^--N, NO_2^--N, NH_4^+-N and WSOC contents were performed daily. When the originally added NO_3^--N was exhausted, a second application of 160 $\mu g\ g^{-1}$ was performed. Later, when no more losses of NO_3^--N were detected, a final addition of 200 $\mu g\ g^{-1}$ of NO_3^--N plus 500 $\mu g\ g^{-1}$ of glucose-C was made, to determine if the interruption of NO_3^--N losses was due to the lack of suitable carbon supply or to other factors.

6.4.2 Results and Discussion

The NO_3^--N content decreased from 217 to 6 $\mu g\ g^{-1}$ and the WSOC also decreased from 203 to 171, after 6 days of incubation (Fig. 6), on day 7, the second addition of NO_3^--N

was made. At this point, the WSOC content had decreased from 171 to 127 $\mu g\ g^{-1}$ since the previous day. After 15 days of incubation, the NO_3^--N decreased to 55 $\mu g\ g^{-1}$, and the WSOC content decreased to 114 $\mu g\ g^{-1}$. Between day 15 and day 16 of the incubation, the NO_3^--N content varied between 55 and 66 $\mu g\ g^{-1}$, in spite of the apparently large WSOC content which varied between 114 and 122 $\mu g\ g^{-1}$. Consequently, at this point it appeared that the indigenous WSOC supply could no longer sustain further NO_3^--N losses. Thus, a third application of NO_3^--N in conjunction with glucose-C was performed, giving a total of 266 and 622 $\mu g\ g^{-1}$ of NO_3^--N and glucose-C, respectively. Within 24 hours the NO_3^--N decreased to 10 $\mu g\ g^{-1}$ and the WSOC content was 219 $\mu g\ g^{-1}$. These results confirmed that the lack of suitable organic carbon supply prevented denitrification from proceeding.

During the incubation, the NH_4^+-N content steadily increased from 13 to 62 $\mu g\ g^{-1}$ during the first 16 days (Table 13). Also, during this period, the presence of NO_2^--N was detected in amounts ranging between 1 and 26 $\mu g\ g^{-1}$.

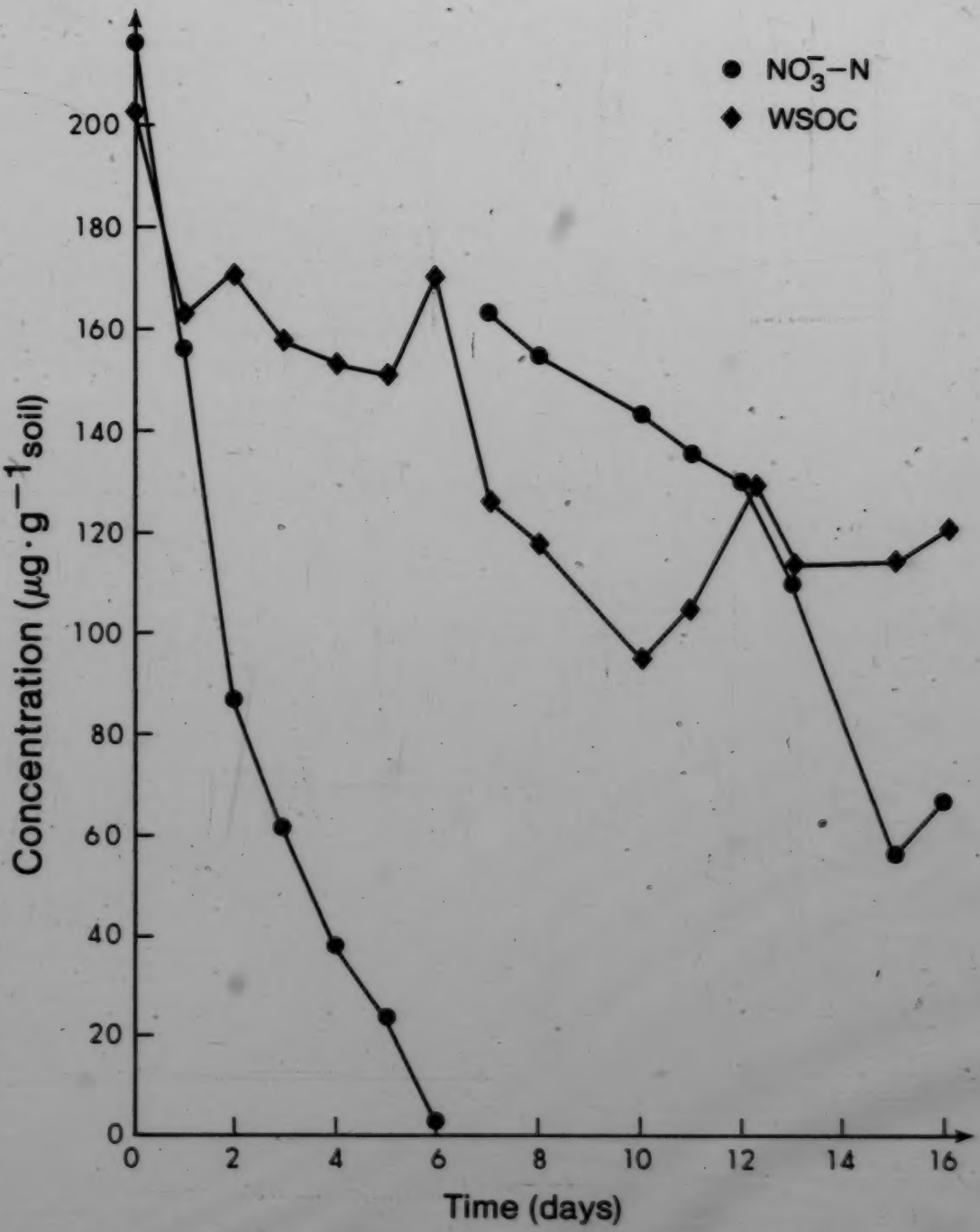

Figure 6. NO_3^-–N and WSOC contents present in anaerobically incubated Malmo soil. Between day 6 and 7, a second dose of NO_3^-–N was added. Between days 16 and 17 glucose–C and NO_3^-–N were added (data shown on table 13).

Table 13. NH_4^+-N, NO_3^--N, NO_2^--N and WSOC contents of Malmo soil samples, when incubated under water saturated conditions, at 28 C in the dark.

Time	NH_4^+-N	NO_3^--N	NO_2^--N	WSOC
days	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
0	13 ± 4	217 ± 12	0	203 ± 13
1	19 ± 2	157 ± 5	26 ± 3	164 ± 3
2	26 ± 1	87 ± 14	14 ± 3	171 ± 15
3	32 ± 1	62 ± 7	13 ± 2	158 ± 8
4	37 ± 2	38 ± 4	3 ± 1	153 ± 6
5	38 ± 1	27 ± 8	<1	152 ± 9
6	44 ± 2	6 ± 1	3 ± 1	171 ± 8
7*	45 ± 4	167 ± 5	21 ± 1	127 ± 12
8	42 ± 3	155 ± 10	5 ± 1	118 ± 9
10	47 ± 2	143 ± 9	<1	95 ± 7
11	51 ± 10	135 ± 3	7 ± 2	105 ± 10
12	51 ± 7	130 ± 7	3 ± 2	129 ± 9
13	52 ± 1	110 ± 9	9 ± 5	113 ± 3
15	58 ± 4	56 ± 9	2 ± 1	114 ± 16
16**	62 ± 6	66 ± 2	4 ± 1	122 ± 14
17	39 ± 5	10 ± 10	17 ± 4	219 ± 16

±indicates standard deviation.
* at this time, 160 $\mu g\ g^{-1}$ of NO_3^--N were added to the soil.
** after obtaining the data for day 16th, 200 $\mu g\ g^{-1}$ of NO_3^--N and 500 $\mu g\ g^{-1}$ of glucose-C were added to the soil.

These results indicate that 321 μg NO_3^--Ng^{-1} were lost within a 15 day incubation period, by which time the WSOC supply had declined to a level which could not sustain further losses of NO_3^--N. As soon as a carbon source was applied, 266 μg g^{-1} of NO_3^--N disappeared within one day. This finding agreed with reports that suggest that denitrification is largely controlled by available C (Limmer and Steel, 1982; Ryden and Lund, 1980 and Rolston *et al.*, 1978).

In the present study, when the losses of NO_3^--N ceased, the WSOC content was still 144 μg g^{-1}, which is higher than the level at which WSOC became limiting in the Breton soil. This fact indicates that the limiting WSOC content is not consistent among soils. Diffusional limitations and the nature of the organic carbon source may vary between soils. Logically, maximal denitrification rates must respond to the number of the electrons per mole of carbon substrate; for example, a mole of hexane will provide twice as many electrons as a mole of glucose. Regarding the nature of the carbon source, Woldendorp(1963), found that the rate of denitrification using glutamic acid as an hydrogen donor, was higher than with glucose. In combination both hydrogen donors increased the rate even more. He obtained similar results with other amino acids (alanine, lysine, vitamin-free casamino acids), which always gave higher denitrification rates than carbohydrates.

6.4.3 Summary

Results reported in section 6.1, 6.2 and 6.3 indicated that NO_3^--N losses in samples of the Malmo and Breton soils were related to the WSOC content. In the present experiment, it was found that up to 321 μg g were lost from air dried samples of the Malmo soil and that NO_3^--N losses ceased to occur when the WSOC content decreased to 144 μg g^{-1}. However, results reported in section 6.1 showed that the limiting WSOC content for air dried samples of the Breton soil was 34 μg g^{-1}, indicating that the limiting WSOC level was not consistent among soils. This was probably due to factors such as diffusional limitations and to the different nature of the organic carbon between the soils. Therefore, the extensive NO_3^--N losses which occurred in the Malmo soil, were limited not only by the quantity of WSOC present in the soil, but also by other factors, which were outside of the scope of this study.

6.5 Effect of the Concentration of NO3-N and NO2-N on Denitrification

Nitrate concentration has been found to influence denitrification and the relative proportion of intermediate compounds. Therefore, the following experiments were conducted to examine the effect of concentration of nitrate, and of nitrate plus nitrite on denitrification in the Malmo and Breton soils. Also, to determine concomitant changes in the WSOC content of the soil, while NO_3^- and NO_2^- losses were

taking place.

6.5.1 Experimental Design

This experiment consisted of three studies, in which samples were incubated following the procedures as outlined in section 3.3. In studies No 1 and No 2, the effect of high concentration of NO_3^--N on denitrification in the Malmo and Breton soils,respectively, were determined. In study No 3, the effect of nitrate plus nitrite on denitrification in the Breton soil was determined. For each study the samples were replicated three times each.

Study No 1

Treatment A was a control, and treatment B received 1468 $\mu g\ g^{-1}$ of NO_3^--N. Air-dried soil samples were used and they were incubated under water saturated conditions for 7 days, at 28 C. Determinations of WSOC content used the persulfate oxidation method (Hu *et al.*, 1972).

Study No 2

In study No 2, pre-incubated Breton soil samples were treated with 345 and 600 μg of NO_3^--N and of glucose-C g^{-1} of soil, respectively. The samples were incubated under water saturated conditions for 10 days at 28 C. Determinations of WSOC content were performed in the Beckman 915-B analyser.

Study No 3

Study No 3 consisted of two treatments:

1. In treatment A, NO_3^--N and NO_2^--N were applied at rates of 271 and 94 $\mu g\ g^{-1}$, respectively.
2. In treatment B, NO_3^--N and NO_2^--N were applied at rates of 200 and 190 $\mu g\ g^{-1}$, respectively.

In this study, pre-incubated Breton soil samples were treated at time zero with the nitrate and nitrite mixtures together with 600 $\mu g\ g^{-1}$ of glucose-C. Samples were incubated under water saturated conditions for 14 days, at 28 C. This study was designed as a completely ramdomized block and analysis of variance was conducted for the NO_3^--N plus NO_2^--N contents remaining during the first three days of incubation. Determinations of the WSOC content were performed with the Beckman 915-B analyser.

6.5.2 Results and Discussion

Study No 1

In the control treatment, the NH_4^+-N content varied between 14 and 68 $\mu g\ g^{-1}$ (Table 14). For the same period, slight changes in the NO_3^--N and NO_2^--N content were detected (ranging between 1 and 12 $\mu g\ g^{-1}$). The WSOC content fluctuated between 980 and 291 $\mu g\ g^{-1}$, during the course of the incubation. After 7 days, the WSOC content was 867 $\mu g\ g^{-1}$. The denitrifying population increased from less than 10 to 1.3×10^5 organisms g^{-1}, after 7 days.

When 1468 $\mu g\ g^{-1}$ of NO_3^--N were applied to the soil, the NH_4^+-N content decreased slightly (from 14 to 11 $\mu g\ g^{-1}$),

after 7 days. During this period, the NO_3^--N content decreased from 1468 to 847 $\mu g\ g^{-1}$. At the same time, the NO_2^--N content increased from 0 up to 209 $\mu g\ g^{-1}$ after 1 day and later it steadily decreased to 86 $\mu g\ g^{-1}$. During the course of the incubation, the WSOC content fluctuated between 980 and 215 $\mu g\ g^{-1}$, however, after 7 days, it was equivalent to 602 $\mu g\ g^{-1}$. No growth of denitrifiers was detected (Table 14).

In general, in both treatments, fluctuations in the WSOC contents were detected , possibly reflecting growth and decay of the microbial population.

Denitrification in the Malmo soil was affected by the initial application of a high dose of NO_3^--N. After 7 days of incubation, 535 $\mu g\ g^{-1}$ of NO_3^--N were lost and 847 $\mu g\ g^{-1}$ remained in the soil, and at the same time an accumulation of NO_2^--N was detected (86 $\mu g\ g^{-1}$), although the WSOC content remained relatively high (602 $\mu g\ g^{-1}$). The same effect occurred in incubation studies on denitrification conducted by Cho and Sakdinan(1978), where NO_2 accumulations were related to the initial concentration of nitrate. Woldendorp(1963), suggested that high nitrate concentration may inhibit denitrification.

Using the values of the WSOC lost per unit of NO_3^--N lost during the total incubation period, the C/N ratio was calculated with the following expression:

$$C/N=[(\text{WSOC lost})/(NO_3^-\text{-N lost})] \qquad [5]$$

Table 14. NH_4^+-N, NO_3^--N, NO_2^--N, WSOC content and population of denitrifiers for initially air dried Malmo soil samples, incubated under water saturated conditions, at 28 C.

Treatment	Time	NH_4^+-N	NO_3^--N	NO_2^--N	WSOC	Denitrifiers
	days	ug g^{-1}	ug g^{-1}	ug g^{-1}	ug g^{-1}	organism g^{-1}
A[1]	0	14 ± 10	11 ± 11	0	980 ± 58	3.2
	1	66 ± 11	12 ± 2	3 ± 2	740 ± 44	7.5 ± 2 x 10^2
	2	42 ± 3	<1	<1	488 ± 48	6.1 ± 2 x 10^4
	3	28 ± 4	0	0	533 ± 61	
	4	51 ± 5	<1	2 ± 1	347 ± 18	
	5	68 ± 5	2 ± 1	4 ± 2	291 ± 24	4.8 ± 1 x 10^4
	7	57 ± 8	11 ± 5	0	867 ± 65	1.3 ± 1 x 10^5
B[2]	0	14 ± 10	1468 ± 74	0	980 ± 58	3.2
	1	22 ± 9	1233 ± 50	209 ± 23	312 ± 25	0
	2	6 ± 4	911 ± 64	118 ± 22	474 ± 54	0
	3	1 ± 1	875 ± 56	198 ± 14	383 ± 64	
	4	26 ± 9	1162 ± 80	178 ± 16	215 ± 26	
	5	17 ± 3	894 ± 75	140 ± 25	240 ± 32	0
	7	11 ± 3	847 ± 68	86 ± 32	602 ± 63	0

± indicates standard deviation.
[1] Treatment A was a control.
[2] Treatment B received 1500 ug NO_3^- g^{-1}.

In this study, the calculated C/N ratio was 1.42, is higher than the theoretical C/N ratio for complete denitrification using glucose as energy source (weight basis=1.07).

Although, NO_3^--N losses were detected when a high dose of NO_3^--N was applied to the soil, no growth of denitrifiers was shown. This was probably due to some bacteria being unable to grow in the NO_2^--N broth used as growth media (Volz, 1977) and to the amount of NO_2^--N present in the soil which may have been toxic to microorganisms. In this context, it has been reported that extracellular NO_2^--N and/or its resultant reactions with organic matter may produce biochemical toxins, a decrease of nutrient availability, or may inactivate microbial enzyme systems in vitro (Castellani and Niven,1975; Riha and Solberg,1973).

The application of a high dose of NO_3^--N had a detrimental effect on the population of denitrifiers, which inhibited the denitrification process to an extent that permitted only partial losses of NO_3^--N and an accumulation and persistance of NO_2^--N, although the WSOC content of the soil remained relatively high.

Study No 2

In study No 2, in which the effect of a high concentration of NO_3^--N in the Breton soil was studied, the NO_3^--N content decreased from 345 to 210 $\mu g\ g^{-1}$ of soil after three days of incubation, and completely disappeared after

10 days of incubation. The NO_2^--N increased from 33 to 93 μg g^{-1} after three days, and decreased to less than 1 μg g^{-1} after 10 days. The NH_4^+-N decreased from 20 to 12 μg g^{-1} after 3 days, and increased to 21 after 10 days. The WSOC content decreased from 578 to 53 μg g^{-1} after 3 days of incubation, and increased to 72 μg g^{-1} after 10 days (Table 15).

In this study, the calculated C/N ratio was 1.33, which is very close to the theoretical C/N ratio for complete denitrification using glucose as energy source (weight basis=1.07). This relationship provides additional evidence that the WSOC fraction is the pool most particularly susceptible to decomposition and is highly related to the denitrification capacity of the soil.

Considering that after three days of incubation only 39% (135 μg g^{-1}) of the originally applied NO_3^--N disappeared from the soil and the amount of NO_2^--N increased from 33 to 93 μg g^{-1}, suggests that denitrification was inhibited during this period by the initially high concentration of NO_3^--N applied to the soil, even though an additional energy source had been provided. However, this effect did not persist after 10 days, where less than 1 μg g^{-1} of NO_3^--N remained in the soil.

Under these experimental conditions, the denitrification process was inhibited by the high dose of NO_3^--N initially applied to the soil.

Table 15. NH_4^+-N, NO_3^--N, NO_2^--N, WSOC content of the Breton soil, amended with 345 μg NO_3^--N g^{-1} and 600 μg glucose-C g^{-1} and incubated under water saturated conditions.

Time	NH_4^+-N	NO_3^--N	NO_2^--N	WSOC
days	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}
0	10 ± 2	345 ± 16	33 ± 5	596 ± 24
1	15 ± 5	303 ± 14	40 ± 4	495 ± 20
2	8 ± 2	269 ± 11	59 ± 6	257 ± 38
3	12 ± 3	210 ± 19	93 ± 7	53 ± 14
10	21 ± 10	0	<1	71 ± 14

± indicates standard deviation.

Table 16. NH_4^+-N, NO_3^--N, NO_2^--N, and WSOC content of Breton soil samples, amended with NO_3^--N and glucose-C and incubated under water saturated conditions.

Treatment	Time	NH_4^+-N	NO_3^--N	NO_2^--N	WSOC
	days	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}
A[1]	0	21 ± 6	271 ± 25	94 ± 15	645 ± 9
	1	18 ± 2	279 ± 16	93 ± 5	613 ± 1
	2	15 ± 1	267 ± 17	79 ± 9	366 ± 21
	3	6 ± 2	152 ± 17	31 ± 8	231 ± 33
	14	27 ± 5	<1	<1	380 ± 24
B[2]	0	16 ± 1	200 ± 10	190 ± 8	665 ± 25
	1	18 ± 1	200 ± 15	159 ± 15	670 ± 13
	2	12 ± 4	195 ± 14	163 ± 1	562 ± 11
	3	13 ± 7	199 ± 16	89 ± 8	343 ± 25
	14	28 ± 1	<1	<1	352 ± 30

[1] In treatment A, NO_3^--N and NO_2^--N were applied at rates of 271 and 94 μg g^{-1}, respectively.
[2] In treatment B, NO_3^--N and NO_2^--N were applied at rates of 200 and 190 μg g^{-1}, respectively.
± indicates standard deviation.

Study No 3

For treatment A, the NO_3^--N content decreased from 271 to 152 $\mu g\ g^{-1}$ after three days of incubation and to less than 1 $\mu g\ g^{-1}$ after 14 days(Table 16). The NO_2^--N content decreased from 95 to 31 $\mu g\ g^{-1}$ after 3 days and to less than 1 $\mu g\ g^{-1}$ after 14 days. The WSOC content decreased from 645 to 380 $\mu g\ g^{-1}$ after 14 days. The ratio of WSOC content that disappeared to the amount of NO_3^--N plus NO_2^--N lost during the incubation period (C/N) was 0.72 (Table 16).

In treatment B, the NO_3^--N content remained unchanged after 3 days of incubation and almost disappeared completely after 14 days of incubation (less than 1 $\mu g\ g^{-1}$). The NO_2^--N content decreased from 16 to 13 $\mu g\ g^{-1}$ after 3 days and to less than 1 $\mu g\ g^{-1}$ after 14 days. The NH_4^+-N content decreased from 16 to 13 $\mu g\ g^{-1}$ after 3 days, but it increased to 28 $\mu g\ g^{-1}$ after 14 days. The WSOC content decreased from 665 to 353 $\mu g\ g^{-1}$ after 14 days. In this case the ratio of WSOC content that disappeared to the NO_3^--N plus NO_2^--N lost during the incubation period (C/N) was 0.80.

In general, the C/N ratios for treatments A and B were very similar with values of 0.72 and 0.80, respectively. These C/N ratios were smaller than that calculated in the previous experiment, which suggested that a greater amount of hydrogen donor, in this case WSOC, was consumed per unit mass of N denitrified, when the N was in the nitrate form than when it was in the nitrite form, which was in agreement

with conceptual considerations of denitrification.

In both treatments, the NH_4^+-N content decreased at the beginning of the incubation. Considering that glucose-C was supplied, some NH_4^+-N inmobilization may have occurred. After 14 days, however, some mineralization occurred and in both treatments the NH_4^+-N content increased to the same level (27 $\mu g\ g^{-1}$).

Results of the present study suggested that the persistance of NO_3^--N and NO_2^--N during the first 3 days of incubation were related to the initial concentration of the NO_3^--N plus NO_2^--N. Statistical analysis of these results showed no effect exerted by the mixture with higher initial content of NO_2^--N.

6.5.3 Summary

It was concluded that the addition of a high dose of NO_3^--N (1500 $\mu g\ N\ g^{-1}$) to the Malmo soil inhibited denitrification and NO_2^--N accumulated. In the Breton soil, the application of mixtures of NO_3^--N plus NO_2^--N (over 300 $\mu g\ N\ g^{-1}$) inhibited denitrification.

Values of the C/N ratios suggested that a greater amount of WSOC was consumed per unit mass of N denitrified, when N was in the nitrate form than when it was in the nitrite form.

7. Conclusions

The results obtained from the experiments previously described permit the following conclusions :

1. Desorption of WSOC from the Malmo and Breton soils can be described by a modified Freundlich isotherm.

2. Parameters of the desorption equation obtained for air-dried and pre-incubated samples of the two soils can be used to determine the portion of the WSOC pool utilized by microbial activity as well as the effect of drying and other perturbations of soil. When air-dried soil samples were incubated at field capacity for seven days, 158 and 45 $\mu g\ g^{-1}$ of WSOC were removed from the Malmo and Breton soils, respectively.

3. Disappearence of $^{15}NO_3^-$-N due to microbial immobilization in a waterlogged soil accounted only for 5% after 15 days of incubation , so that denitrification was considered the main mechanism of $^{15}NO_3^-$-N losses.

4. The Malmo and Breton soils had different capacities to denitrify and they were closely related to their different WSOC contents, which were significantly different between the two soils. The lower WSOC content at which denitrification occurred in the Malmo soil was 144 $\mu g\ g^{-1}$, however, in the Breton soil the WSOC content at which denitrification ceased was 34 $\mu g\ g^{-1}$, indicating that the limiting WSOC content was not the same among soils.

5.- The addition of a high dose of NO_3^--N (1500 $\mu g\ g^{-1}$) to the Malmo soil inhibited denitrification and NO_2^--N

accumulated. In the Breton soil, the application of mixtures of NO_3^--N and NO_2^--N (over 300 $\mu g\ g^{-1}$) inhibited denitrification as well. In these studies, values of the C/N ratios suggested that a greater amount of WSOC was consumed per unit mass of N denitrified, when N was in the nitrate form than when it was in the nitrite form.

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8. Appendix

Table A. General characteristics of the Malmo and Breton soils.

Soil Order	Chernozemic	Luvisolic
Soil Series	Malmo	Breton
Management	Alfalfa Sod	Stubble
Total C (%)	5.30	1.20
OM (%)	9.23	2.08
pH	6.20	6.44
Total N (%)	0.54	0.12
Total P (%)	0.10	0.06
Total S (%)	0.09	.02
Na[1] (meg/100g)	0.24	0.19
K[1] (meg/100g)	0.70	0.44
Ca[1] (meg/100g)	36.11	15.28
Mg[1] (meg/100g)	6.88	1.70

[1] Exchangeable Na, K, Ca and Mg.

Table B. WSOC contents and standard deviation of soil extracts obtained by using different volumes of extractant per unit soil mass, analyzed by the Difference Method and by the Acid Sparge Method for the Malmo and Breton soil.

Volume of Extractant/Soil Mass	Water Soluble Organic Carbon (ug g^{-1})			
	Malmo Soil		Breton Soil	
ml g^{-1}	Difference Method	Acid Sparge Method	Difference Method	Acid Sparge Method
2	423 ± 36	417 ± 6	100 ± 2	120 ± 6
5	601 ± 18	500 ± 10	139 ± 6	154 ± 6
10	764 ± 9	778 ± 10	310 ± 7	177 ± 3
100	2126 ± 56	866 ± 76	976 ± 50	536 ± 15

± indicates standard deviation.

Table C. Calculation of % Abundance and % Excess ^{15}N from mass spectrometer determinations.

$$\% \text{ Abundance} = 100/((272((\text{Ratio}^{1}\text{Ref} + \text{Read Ref})/(\text{Ratio Sam} + \text{Read Sam} + \text{offset}))) + 1)$$

$$\% \text{ Excess } ^{15}N = \% \text{ Abundance} - 0.365^{2}$$

[1]Where Ratio is the ratio of ^{14}N and ^{15}N

[2]The natural abundance of ^{15}N in the atmosphere was taken as 0.365% and used in these calculations.

Table D. Calculation of the labelled ^{15}N remaining in the soil samples in the form of NO_3^--N, NO_2^--N, NH_4^+-N and Total N (NO_3^-+NO_2^- included).

$$\text{Labelled fertilizer } (\mu g\ N\ g^{-1}) = \frac{\%\text{Excess Sample}}{\%\text{excess of fertilizer}^{3}} \times \text{Amount of N in sample}^{4}$$

[3]Where %Excess of fertilizer is a constant = 1.3556.

[4]Nitrogen in the sample ($\mu g\ g^{-1}$)